THE
AMERICAN NATURALIST

VOL. LXXII *May–June, 1938* No. 740

SEX DIFFERENTIATION[1]

ABERRANT FORMS IN DROSOPHILA AND SEX DIFFERENTIATION

PROFESSOR J. T. PATTERSON
UNIVERSITY OF TEXAS

OUR views on sexual differentiation have undergone many changes since the beginning of the present century. Following the discovery of the sex chromosomes, it was assumed that the sex of the individual in forms which have the XY type of sex determination was dependent upon the presence of one or two X-chromosomes. According to this assumption, zygotes which had received two X-chromosomes at the time of fertilization developed into females, while those which had received a single X-chromosome developed into males.

But this explanation was found to be inadequate, particularly in the face of many newly discovered facts which soon followed. The development of the gene theory, coupled with important discoveries along the lines of sex hormones, sex reversals and intersexes, all served to emphasize the inadequacy of so simple an explanation. It was not surprising then that Goldschmidt and Bridges, upon discovering intersexes in Lamantria and Drosophila, respectively, should have formulated theories on sexual differentiation which would be in accord with the modern theory of genetics. In accordance with the title of this

[1] A symposium given before the American Society of Zoologists and the Genetics Society of America at Indianapolis, Ind., the American Association for the Advancement of Science, December 28, 1937. A fourth paper, entitled "The Rôle of Hormones in the Differentiation of Secondary Sex Characters," by Dr. L. V. Domm, will be published elsewhere.

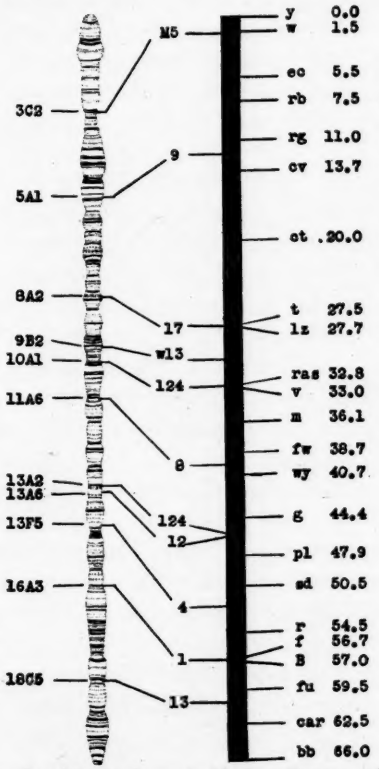
address, I shall restrict my discussion to the conditions found in *Drosophila.*

In 1921 Bridges discovered triploids and intersexes in *Drosophila melanogaster,* and upon the basis of his study of these and certain other variant forms, he has developed what is known as the theory of genic balance. This theory is so well known that it will not be necessary to state it in other than the briefest terms. His main conclusion was that, "A preponderance of genes that are in the autosomes tend toward the production of male characters; and the net effect of genes in the X is a tendency to the production of female characters." On the basis of this assumption, sex is determined by a balance between the X-chromosomes and the autosomes, and any change which modifies the normal ratios of 2X:2A in the female, or 1X:2A in the male, will upset the balance and result in the production of abnormal flies. Thus a change to 2X:3A results in the formation of the sterile triploid intersex, or a change to 3X:2A produces the sterile superfemale.

Bridges's theory implies a multiple factor system, but there is another possible interpretation of the facts concerning sex determination in *Drosophila.* They may be explained on the assumption that there is a single female sex-differentiator in the X-chromosome and that all other genes located in this element are not concerned with sex. In discussing these two alternative assumptions, Morgan (1926) pointed out that we might hope to discover something about the genes that influence sex if the X-chromosome became fragmented in such a way as to reveal the location of sex genes, if they really exist. It was not, however, until Muller (1927) discovered that x-rays would break the chromosome that a method for testing these assumptions was available. In his first full paper reporting this discovery, Muller (1928) pointed out that locus rearrangements, including translocations, induced by x-rays could be used for studying the problem of the locus of the sex-differentiator.

It was evident that if there were a single female sex-

differentiator located in the X-chromosome, some one of the fragments, into which the chromosome could be broken by x-rays, would contain its locus. By testing the effect



TEXT-FIG. On the right is shown in black a diagram of the genetic map of the X-chromosome. The loci of twenty-three mutant genes are indicated, beginning with that for yellow at the distal or left end and terminating with the locus of bobbed at the proximal or spindle-fiber end. To the left of this map are shown the points of breakage of ten different translocations, which were used in the studies referred to in the text. The exact points of breakage are also shown on the salivary-gland chromosome. The left- and right-hand parts of a broken X in any translocation are designated by the letters L and R, respectively. A duplication for any sector was obtained by using the parts of two translocations which overlapped that sector. Thus, the duplication for the interval M5-9 was obtained by using M5 R plus 9 L. The corresponding deficiency was obtained by using M5 L plus 9 R. The numbers on the extreme left show the points of breakage in terms of Bridges' 1935 map.

on sexual differentiation of adding and subtracting each fragment to or from the normal chromosomal complex of diploid males and females, it would be possible to detect the presence of such a factor and to determine its approximate location on the chromosome. If, on the other hand, there were many "female-tendency" genes scattered along the X-chromosome, the addition or subtraction of any fragment would only partially transform the sex of the individual.

Dobzhansky and Schultz (1931, 1934) tested these alternative assumptions by studying males, females and intersexes which carried duplications or deficiencies for different regions of the X-chromosome. They were especially interested in comparing triploid intersexes of the composition 2X: 3A with their sibs 2X: 3A plus different fragments of the X. They found that certain long duplications produced extreme female-type intersexes, which in some cases were transformed into fertile hypotriploid females.

In our own work on *D. melanogaster,* we were interested in studying the effects of aneuploidy of the X-chromosome, as revealed in hyperploid males and hypoploid females. We also studied this phenomenon in hyperploid females and in sex-mosaics or gynandromorphs. In hyperploid flies, the extra chromosomal fragment is called a "duplication," while in hypoploid flies the missing section is referred to as a "deficiency."

GYNANDROMORPHS

The first approach to the problem was made in 1928 in a series of x-ray experiments which had as their object the production of gynandromorphs. It was believed that longer duplications and deficiencies might survive in mosaic than in non-mosaic flies. Morgan and Bridges (1919) had shown that a majority of the gynandomorphs occurring spontaneously in *Drosophila* could be accounted for on the basis of Morgan's (1914) assumption that one of the X-chromosomes in an XX-zygote had been eliminated from an early cleavage cell. Such mosaics would

have two X-chromosomes in the tissues of the female parts of the fly and only one X in the tissues of the male parts. According to the hypothesis, the size of the male area in the mosaic would be determined by the cleavage division at which the elimination occurred. If it takes place at the first division, the male area will constitute approximately one half of the fly; if at the second division, it will be limited to about one fourth of the individual, and so on throughout the succeeding divisions. Once established, and with but few exceptions, the variant area develops under the influence of its own genetic constitution, that is, it is self-differentiating.

The object of the experimental tests was to determine what effect the addition or subtraction of a given section of the X-chromosome would have on the sexual differentiation of a mosaic area. The usual experimental procedure was followed of x-raying flies which in the main carried wild-type genes, and then crossing the treated flies to individuals with X-chromosomes having a series of well-spaced recessive genes to serve as markers. In event the x-radiation has caused the elimination of a section of the X-chromosome, the deficiency can be detected by the phenotype of the variant area in the F_1 fly, and the marker genes will indicate just what part of the X has been lost. Moreover, the character of the mosaic area in any of the main sexually differentiated parts of the fly will show whether or not a postulated, major sex gene had been removed. Experience has shown that the gametic chromosome must in effect be in the two-strand stage at the time of treatment, if the variant area is to receive a broken daughter chromosome, otherwise, a mosaic fly will not be produced.

The most interesting results were obtained from experiments in which the males had been treated. We obtained in this series more than one hundred and fifty gynandromorphs in which the mosaicism could be attributed to the effects of x-rays. The marker genes in the untreated maternal X indicated that in fifty of these flies the male parts carried a broken X-chromosome, in addition to the one received from the mother. As was to be expected, the

treated paternal X-chromosome had been broken at different points in different mosaics. In a majority of cases the missing section was from the middle region of the chromosome, and on the basis of genetic tests of fertile hyperploid males, this would indicate that the extra fragment in the male tissue of the mosaic was composed of the united ends of the broken X.

The main point of interest was to determine the longest left- and right-hand portions of the duplicating fragment that would survive as hyperploid male tissue. In three gynandromorphs the left-hand break that produced the deletion in the daughter chromosome had occurred to the right of the locus of miniature, and in one of these the break had taken place between miniature and furrowed. Altogether, seven gynandromorphs were produced in which the right-hand break in the daughter chromosome had occurred to the left of the locus of forked. Thus, the results obtained from a study of artificially produced gynandromorphs showed very clearly that the sections from yellow to miniature of the left end, and from forked to the spindle fiber of the right end of the X-chromosome, do not contain a major female sex-differentiator. But this left the middle section of slightly over twenty map units still untested, except the results showed that whenever this section was absent from the diploid complex the phenotype of the variant area was male in character, and that whenever present the variant area was female in character. Inasmuch as there was no effective way of isolating and testing this middle section in gynandromorphs, it was decided to approach the problem by other methods.

HYPERPLOID MALES

There were found among the F_1 flies, obtained in the experiments on gynandromorphism, a number of aberrant males. These were not mosaic flies, but X-hyperploids having a genetic constitution similar to that of the male parts of a gynandromorph carrying an extra fragment of a broken chromosome. They differ from the latter in that the breaks producing the fragment must have occurred while the chromosome was still in the single

strand stage, or else both completed daughter chromosomes had been broken at the same level. As in the gynandromorphs, the duplicating fragment in these males was composed of the united ends of the treated X, with the middle section lost, and the length of the fragment likewise varied in different males.

The longest duplication composing the right-hand portion of the fragment included the forked-spindle fiber section, the break having taken place between the loci of forked and garnet. We have found evidence which indicates that a short section lying just distal to the locus of forked does not change sex to femaleness when duplicated. A duplication including the rudimentary-forked section was obtained and tested, and found to be ineffective. The left-hand portion of the fragment was found to be very short in most cases. In many individuals the break had occurred between yellow and scute, and in another large group it had taken place between scute and white. In but a single detectable case was it found that the break had occurred to the right of the locus of white. In this individual the break had taken place between the loci of white and crossveinless, which was the next marker gene to the right of white. Further tests to determine the exact point of breakage were not possible, because the aberrant male has no Y-chromosome and is therefore sterile.

A comparison of the aberrant male with the gynandromorph shows that the tissues of the former will carry nearly as long a duplication from the right end of the X-chromosome as will the tissues of the male parts of the latter. But this is not true for duplications from the left end of the X, for the results show that the male parts of the gynandromorphs will tolerate a much longer section (*y–m*) than was found to be true for the aberrant males (*y–w*). Presumably, this is due to a favorable influence exerted by the normal diploid half of the mosaic upon the viability of the hyperploid male half.

It was desirable to make tests with duplications which were not composite in nature. Even in the few aberrant male, in which the entire left end of the X appeared to be absent from the fragment, the possibility remained that

a very small piece lying distal to the left-hand marker (yellow) might be present in the duplication. One of the best ways to avoid this difficulty is by the use of translocations. This procedure has the further advantage in that a Y-chromosome can be introduced into males carrying the translocated fragment, and thus if the male is fertile, one can determine more accurately the length of the duplication by genetic tests.

It was therefore decided to secure as many translocations as possible, and an x-ray experiment, carried out in 1933 in cooperation with three junior colleagues (Stone, Bedichek, Suche, 1934) gave a total of 1,992 apparent translocations, of which fourteen involved the X and IV chromosomes. The X–IV translocations were desired because most translocations are mutuals, and the small piece of the IV attached to the X causes less disturbance in the X-hyperploids than the usually longer piece from one of the larger autosomes. Three similar stocks were already available (two found by Muller and one by Stone) and another was later obtained by Mr. George Mickey. Ten of these X–IV translocations constituted the stocks upon which we have based a study of the effect of aneuploidy on sexual differentiation as it is revealed by X-chromosome hypoploidy in the female and hyperploidy in the male (see text-fig. for nature of these stocks and points of breakage).

In one series of tests hyperploid flies were produced by adding duplications of varying lengths from one or the other end of the X-chromosome. We were interested in studying the effect of the various duplications on the phenotype, the viability and the fertility of the hyperploid females, but incidentally secured four types of hyperploid males, which were all that survived. These four types, together with three others already described in the literature, made it possible to determine the effect on sexual differentiation of duplications from the ends of the X-chromosome. Four of these were duplications from the left end and three from the right end of the chromosome.

The four duplications from the left end which survived as hyperploid males were as follows: The shortest dupli-

cation tested included the yellow-white section (M5 L), and while the males carrying this fragment were only 7.2 per cent. viable, yet those that did survive were 74.0 per cent. fertile. The next duplication represented the yellow-echinus section, and these males were sometimes fertile (Patterson, 1932). The next longest duplications (*y–rb* section) are from two translocations (X–III and X–II) described and tested by Dobzhansky and Schultz (1934), who states that in each case the point of breakage occurred between ruby and crossveinless. They found among thousands of males free from the duplication only six hyperploid males, all of which were sterile. The longest (probably) duplication which survived as hyperploid male included the yellow-rugose section (9 L). A single, sterile, abnormal, hyperploid male was found among many males free from the duplication.

The duplications from the right end which survived as hyperploid males were as follows: Males carrying the carnation-spindle fiber duplication (13 R) were highly viable, but were found to be only 20.0 per cent. fertile. Males with the *B*-spindle fiber duplication (1 R) were viable but sterile. Our next longest duplication (4 R) did not survive in males, but Dobzhansky and Schultz found one (their duplication 138), which included the rudimentary-spindle fiber section, and males carrying this duplication were sometimes viable but completely sterile.

The method of adding duplications from the ends of the X did not provide means for testing the effects of hyperploidy for some forty-five map units in the middle region of the chromosome. Males hyperploid for duplications long enough to include the mid-section simply do not live. It was therefore necessary to use a series of short duplications. In order to obtain such a series we followed the plan, first suggested by Muller (1930), of combining two parts of different translocations in such a way that the left part of one will "overlap" the right part of the other. By making proper selections from among the several translocations, we were able to duplicate the entire X-chromosome in ten short intervals. The method

of using short sections in tests for a postulated female sex-differentiator has certain inherent advantages over the use of long duplications. We have shown (Patterson, Stone, Bedichek, 1937) that the effect of a long duplication does not allow one to predict the effect of its component parts, if these are represented in short sections.

We found that males survived with a duplication for any one of nine of the ten intervals. Not only were the hyperploid males viable, but they were also fertile, although in two cases the per cent. fertile was very low. Males with interval 9–17 were only 1.1 per cent. fertile, and those with interval 12–4 showed but 2.0 per cent. fertile. The per cent. fertile for the other duplications varied from 20.0 for interval 13 R to 91.0 for interval 4–1. The single section, which failed to survive as hyperploid males includes the loci of wavy and garnet among the better known genes. No male survived as X duplication for the interval 8 and 12, although numerous efforts have been made in attempts to secure viable zygotes hyperploid for this interval.

We have another duplication which covers the major portion of the interval 8–12. This is the vermillion-garnet sector of translocation 124, which was deleted from the X and inserted into the II chromosome. Experimental results showed that flies with one X plus this duplication almost invariably die before the larvae emerge from the eggs. Recently, however, we have succeeded in bringing to maturity four such flies, all of which were males. One of these males was abnormal and the other three were sterile. Had a larger number been obtained, it is possible that some few of these males might have been fertile. In the hyperploid male test all regions of the X-chromosome have been covered by short duplications, except a small section lying between the loci of garnet and pleated, and occupying the position of bands 13A2 to 13A6 of Bridges's 1935 map of the salivary-gland chromosome (see text-fig.).

HYPERPLOID AND HYPOPLOID FEMALES

Females hyperploid for each of the ten intervals were found to be viable and to some extent fertile. They were

somewhat more viable and more fertile (except for interval w13–8) than the corresponding hyperploid males. The hyperploid females did not give any critical evidence concerning the presence or absence of a primary sex-factor in the X. The hypoploid females furnish a much more critical test. These can be produced for the several intervals by selecting from two translocations right and left parts which fail to meet, and thus create short deficiencies in the heterozygous females. Unfortunately, only four types of hypoploid females survived the tests. These were for intervals M5–9, 17–w13, w13–8 and 13R. All were fertile except females deficient for interval w13–8. Dobzhansky and Schultz (1934) also obtained females deficient for this same general region, but they did not state whether such flies were fertile.

Since diploid females deficient for six out of ten of the intervals did not survive, the hypoploid test for sexuality could not be applied to a considerable portion of the X-chromosome. Some parts of the untested portion have been covered by deficiencies created in mosaic females by means of x-rays. Hypoploid tissue will survive with longer deficiencies in mosaic than in non-mosaic flies, and at the same time show female characteristics. Various mosaic females obtained in this way showed losses of different lengths of the left end of the treated X-chromosome. The longest deficiency detected in a mosaic female included the section from yellow to garnet. Such half-and-half mosaics are nearly always sterile—a condition probably due to the effects of aneuploidy.

Discussion

As was pointed out at the beginning of this address, two main theories of sex determination have been developed. One of these assumes that sex is determined by multiple factors, the other implies that sex is determined by the interaction of one or a few main genes, either with or without modifiers. It is known that both types of gene systems are operative, and in dealing with any particular form it becomes necessary to determine experimentally which system really exists. In a recent publication (1937) we

have discussed various aspects of these systems, and I shall summarize from this a few of the more important points.

In races of *Lamantria dispar,* Goldschmidt (1934) has shown that sexual differentiation is controlled by a principal male-tendency gene, M, in the X-chromosome, and a female-tendency influence, F, in the cytoplasm. In pure races the net effectiveness of F and M are so balanced with each other that F M is a female (the digametic sex) and F MM is a male. Certain interracial crosses produced diploid intersexes, which ranged from sex reversal males to sex reversal females, due to the fact that the potency of F and M varies in different races.

For *Habrobracon juglandis* Whiting's (1935) theory of sex determination implies a single factor system, but Snell (1935) has offered what amounts to a multiple factor system for this form. At present it is difficult to determine which of these two alternative assumptions is the correct one for sex determination in the Hymenoptera.

Both the one gene and the multiple gene theories have been advanced to explain sex determination in *Drosophila.* Dobzhansky and Schultz (1934) have carried out an extensive series of experiments with the view of settling the question. They especially emphasize the results obtained with duplications that shifted the balance in triploid intersexes. They argue that the threshold for change in direction of sexual differentiation is higher in diploid flies than in triploid intersexes, and, consequently, if a number of genes control sex, a short duplication need not be expected to produce a reversal in sex. Their general conclusion was that "The sex-determining rôle of the X-chromosome of Drosophila is due to a cooperative effect of numerous female modifiers located in all regions of the chromosome except the inert region."

It might be well, in this connection, to mention the fact that two investigators have reported the discovery of genes, located in the autosomes, which influence sexual differentiation. Sturtevant (1920) has reported the discovery of a recessive autosomal mutant gene in *D. simulans,* which, although not affecting the normal chromo-

somal mechanism, did modify the end result so that females were changed into diploid intersexes. More recently, Lebedeff (1934) has reported several mutations which affect sex determination in *D. virilis*. The principal gene is a recessive mutation, *ix*, located in the third chromosome. When *ix* is homozygous, diploid flies with two X-chromosomes are phenotypic males. There are several modifiers of this gene which cause the action of the gene to be less pronounced, resulting in sex intergrades.

In our own work on *D. melanogaster* we have been concerned with the question of whether or not the X-chromosome contained a single female sex-differentiator, and the main purpose of the various experiments was to test this question. We have assumed that the best test for such a factor was to be found in a study, either of hyperploid males carrying duplications of relatively short sections of the chromosome, or else of hypoploid females with deficiencies for these same sections. The hyploid females proved to be of limited value, because of their failure to survive with deficiencies for a majority of the sections. In contrast to this condition, the hyperploid males were viable for all except a very short section lying between the loci of garnet and pleated. Moreover, each type of hyperploid male was fertile with the exception of that carrying Duplication 124, where the number obtained was too few to furnish an adequate test for fertility.

The males hyperploid for the different short duplications showed no intersexual or female characteristics, and the females surveying hypoploid for four different regions showed no tendency toward maleness. Such phenotypic abnormalities as were revealed in these aneuploids can not therefore be interpreted as positive shifts in the sex of the organism. These abnormalities, as well as the infertility and lack of vigor found in certain types, are in our judgment due to the effects of aneuploidy. We conclude that a female sex-differentiator of the type postulated can not be present in any of the duplications which survived as male hyperploidy.

This leaves for consideration the small section covered

by Bands 13A2 to 13A6. Every attempt to obtain males carrying a duplication which included this short segment has so far failed. Nor have we been able to secure females with the corresponding deficiencies. So far as we know such flies have not been obtained by other workers. This negative evidence might be regarded as indicating the presence of a major sex gene for this region. But such evidence is not critical, because hyperploid males for certain other regions were practically inviable.

LITERATURE CITED

Bridges, C. B.
1921. *Science*, n.s., 54: 252–254.
1932. Chap. III, "Sex and Internal Secretions," 55–93, Baltimore.

Dobzhansky, Th.
1935. *Jour. Genet.*, 31: 155–156.

Dobzhansky, Th., and Jack Schultz
1931. *Proc. Nat. Acad. Sci.*, 17: 513–518.
1934. *Jour. Genet.*, 28: 349–386.
1934. AM. NAT., 68: 190–191.

Goldschmidt, R.
1934. *Bibliographia Genetica*, 11: 1–186.
1935. *Jour. Genet.*, 31: 145–153.

Lebedeff, G. A.
1934. *Proc. Nat. Acad. Sci.*, 20: 613–616.

Morgan, T. H.
1914. *Proc. Soc. Exp. Biol. and Med.*, 11: 171.

Morgan, T. H., and C. B. Bridges
1919. *Carnegie Inst. Wash.*, 278: 1–122.

Muller, H. J.
1927. *Science*, n.s., 60: 84–87.
1928. *Ztsch. f. indukt. Abstammungs-u. Vererbungsl.*, Sup. Bd., 1: 234–260.
1930. *Jour. Genet.*, 22: 299–334.

Patterson, J. T.
1931. *Jour. Exp. Zool.*, 60: 173–211.
1932. *Genetics*, 17: 38–59.

Patterson, J. T., Wilson Stone, Sarah Bedichek and Meta Suche
1934. AM. NAT., 68: 359–369.

Patterson, J. T., Wilson Stone and Sarah Bedichek
1935. *Genetics*, 20: 259–279.
1937. *Genetics*, 22: 407–426.

Punnett, R. C.
1933. AM. NAT., 67: 473–476.

Sturtevant, A. H.
1920. *Science*, n.s., 51: 325–327.

Whiting, P. W.
1935. *Jour. Hered.*, 26: 263–277.

HORMONAL CONTROL OF SEX DIFFERENTIATION[1]

R. K. BURNS, JR.
DEPARTMENT OF ANATOMY, THE UNIVERSITY OF ROCHESTER, SCHOOL OF MEDICINE AND DENTISTRY

I. INTRODUCTION

THE knowledge that the gonads exercise some form of remote control over sex structures and characters of all categories is not new. The effects of castration on the animal body have been well known since ancient times. However, it is usual to credit Berthold (1849) with the first experimental demonstration that substances originating in the gonads and distributed with the circulating blood are necessary for the maintenance of structure and function in the reproductive system. Concrete application of the hormone theory to the development of sex is relatively recent, dating from the first satisfactory analysis of the freemartin problem arrived at independently by Lillie, and Keller and Tandler in 1916.

This milestone marked at once the end of a problem of long standing and the beginning of a period of experiments on the mechanisms of sex differentiation, differing widely in method but having in common the theoretical conception developed for the freemartin—that sex-specific hormones are produced, circulate with the blood and act upon the appropriate embryonic structures during the plastic stages of development.

It is unnecessary now to review the freemartin case, for both facts and argument are well known. However, in arriving at a satisfactory general theory many secondary but nonetheless important questions were developed. Many of these are critical for the further extension of our theoretical structure. We may mention (1) the problem of the nature of the sex-differentiating hormones and their

[1] Read at a joint symposium on "Sex Differentiation" of the American Society of Zoologists and the Genetics Society of America, American Association for the Advancement of Science, Indianapolis, December 28, 1937.

relation to "inductor substances" and adult hormones; (2) the manner in which hormones act on the embryonic primordia; whether (a) stimulatory or inhibitory, or (b) direct or indirect (*i.e.*, whether the mediation of other organs or tissues is requisite); (3) quantitative aspects of hormone action, such as the relation of concentration and duration of action to the effects obtained.

Inseparable from these problems of physiology are others relating to the embryonic constitution and the inherent capacities of the primordia composing the embryonic sex complex. Here we may mention (1) the time of appearance and periods of differentiation of the various primordia; (2) the potencies of these components as determined by inherent capacity and previous conditioning.

II. Hormones and Sex Reversal

Before proceeding with a discussion of these topics let us quickly survey some of the pertinent evidence, in particular that arising from experimental sex reversal. An historical review is not possible; we may, however, mention the experimental methods which have given us most of the data in question: (1) Parabiosis, limited to amphibians so far as sex reversal is concerned; (2) host-graft experiments of several types, notably embryonic transplantations of undifferentiated amphibian gonads (Humphrey) and chorio-allantoic grafting in the chick; (3) castration experiments; (4) the administration of prepared hormones, a comparatively recent development. By way of a general picture derived from this work, we may say: (1) Reversal of sex may proceed in either direction; ovary may undergo reversal to testis, or testis to ovary; (2) the whole range of the reversal process has been demonstrated, from an entirely negative response (parabiosis in Bufo) to complete morphological and functional reversal; (3) incomplete reversal (or absence of any effect) is attributable to differences of hereditary constitution or to various experimental conditions which

modify the processes of differentiation to a greater or lesser degree.

In considering this work we may fairly assume, for the most part, that positive results are due to hormone action in the usual sense. Either the effects are produced at considerable distances, as in most parabiosis experiments; or we are dealing with the injection of substances having known specific effects. In some cases we are faced with experimental conditions in which another form of humoral transmission—"induction"—seems the best device available to explain the results. Where evidences of hormone action are lacking, as in chorio-allantoic grafting or parabiosis in Bufo, we may assume for the time that the sex-differentiating principles, during the stages of development in question, are not sufficiently concentrated to be effective at a distance, although active locally in the manner of inductors.

In the following discussion we shall keep as closely as we may to the problems arising from the interaction of sex-differentiating substances with the various rudimentary parts comprising the embryological foundations of sex.

III. The Embryonic Sex Complex

Lillie (1917) was careful to point out that in the freemartin the male hormone does not cause the development of any structure not represented embryologically in the normal female, and has since emphasized with others the bisexual organization of the embryo as the basis of sex reversal. The processes of reversal follow a pattern normal for the other sex. This means that the mechanisms of normal differentiation and of sex reversal are essentially the same. Specific hormones act upon definite embryonic rudiments, which react in accordance with their special abilities. Upon the intrinsic capacity of each rudiment, and its previous differentiation or "conditioning," depends the kind of response and, in part, its degree. In experimental sex reversal the hormonal factor in these reactions is altered; the embryonic base remains the same.

Therefore a consideration of the status of the various parts comprising the primary sexual equipment is in order.

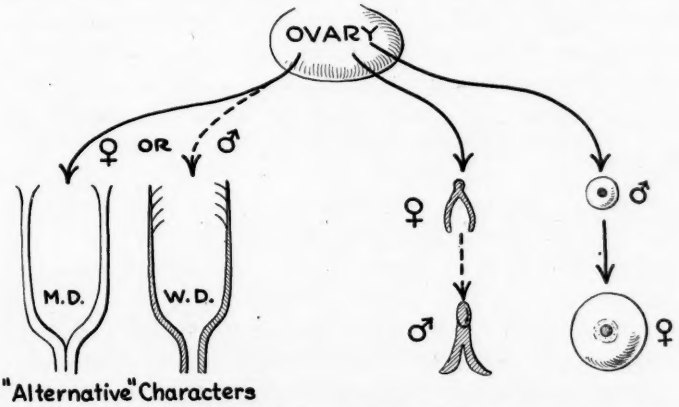
The primitive sexual apparatus of the vertebrate embryo consists of a variety of rudiments or primordia arising at different times during development, having their primary determination in the sex genotype. Collectively they may be termed the *embryonic sex complex.* Classifications propose usually two main categories of characters, *primary* and *secondary,* with various subordinate groups. This type of classification chiefly envisages the final status of the various structures in terms of function; it is not particularly useful from the view-point of developmental physiology. Let us take again the main fact, that every embryo during the indifferent period of its development is completely bisexual, containing all the primordia necessary for the realization of either sex. Upon this series of paired homologues the sex-differentiating hormones have been conceived to act in a selective fashion.

As a matter of fact the individual parts of this complex do not exclusively characterize one sex or the other, as the idea of selection might imply. In the *definitive sex equipment* they may be variously represented. Certain structures, such as mullerian duct or wolffian duct, as a rule become expressed in one sex only; in the other they survive as mere vestiges. Other characters, such as the parts of the external genitalia, form a part of the definitive sexual equipment of both sexes. The difference is essentially a quantitative one; the extent to which the character is expressed is relative.

On this basis we may (as a matter of practical importance) recognize two types of embryonic rudiment: those which have their final expression in one sex only, and those which have a definitive expression in both sexes, but in different degree. For convenience let us call characters of the first category *alternative characters,* signifying merely that in their final expression they characterize one sex or the other. In the development of characters of this

type no doubt hormones exert a selective action in the usual sense, and the selection is exercised between non-homologous parts. For the other type of character we can scarcely conceive that there is a choice in the same sense; for an identical structure (homologous part) develops in both sexes. This is obviously so for the parts of the external genitalia. Characters of this type may, for lack of a better term at the moment, be called *non-alternative*. Secondary sex characters in general also belong to this category, but since usually they are differentiated late in development, the distinction is less important.

Within the differentiating gonad two histological elements are represented, which have opposite developmental capacities. These components are primitive cortex and medulla, having the value respectively of ovary



RELATION OF ALTERNATIVE AND NON-ALTERNATIVE CHARACTERS TO GONAD DIFFERENTIATION

FIG. 1. Scheme illustrating the relations of the primordia of the definitive sex equipment to gonad differentiation. Mullerian duct and wolffian duct are *alternative characters,* having their definitive expression in one sex only. The parts of the external genitalia have definitive expression in both sexes (*non-alternative characters*) and are derived from identical rudiments. They differ chiefly in a quantitative way. Mammary glands as representative secondary sex characters are also non-alternative, but remain sexually indifferent for a long time.

and testis. This condition is familiar in the consecutive production of two proliferations of sex cords—cortical and medullary. The expression of these components may depend on sex genotype, group or species, stage of differentiation or, in birds, on the strange lateral asymmetry which affects the constitution of the gonads. In normal development and in reversal of sex, cortex and medulla comprise alternative possibilities leading to ovary or testis as the case may be; and the same situation prevails as in the case of the gonoducts.

The distinction developed may be important under experimental conditions, where both sex-differentiating hormones may be acting at once or in close sequence. For instance, *alternative characters* might be expected to develop simultaneously with less "conflict of interest," so to speak, than for identical parts which develop differently in the two sexes. In the latter case previous conditioning of a part is of prime importance. The distinction proposed may, of course, be called descriptive rather than analytical; it may still be useful if problems involving the action of hormones on particular characters are made clearer. The situations pictured may be illustrated schematically in Fig. 1.

IV. Mode of Action of Hormones

We come next to the question of the manner in which hormones exert their effects on a particular rudiment or set of structures. For convenience in referring to structures representing one sex type or the other, we shall use the terms *homotypic* and *heterotypic,* so designated with reference to the type of hormone acting or presumed to be acting.

From a purely theoretical point of view various modes of action are possible. We may mention some now accepted or under consideration. If we assume that each hormone in some manner favors development of appropriate (homotypic) parts it would be simple to suppose that each acts directly and in a positive manner. Unfor-

tunately this is not the case for all parts, though probably true for some. On the other hand, some hormone effects are exerted indirectly; the hormone by inhibiting one part or tissue may release another from domination. This indirect mode of action may be called *activation through release.* It particularly fits experimental conditions, where a hormone from without is interfering with the action of the normal differentiation mechanism. But it must be borne in mind that a hormone may act in this manner on one part and in a direct manner on another; and that direct action may be stimulatory or inhibitory. Finally, we must concede that some parts of the sex complex may develop largely or even entirely without the intervention of hormones, in the manner of somatic characters.

Normal development offers limited opportunity for settling such points, but under experimental conditions where, by a variety of methods, hormones of opposite sex type have been introduced, we may observe at once their action on heterotypic primordia, and the effects when both hormones are present at the same time. Let us take up the various parts separately, referring first to the gonad.

The Gonad

Here we are faced with special problems, for the gonad components are, to the best of our knowledge, at once the producers of sex differentiating substances, and a dual system which reacts to its own products. We have also the question whether these products, conceived to behave locally as embryonic inductors (Witschi), are identical with the embryonic hormones or are distinct substances whose province is the gonad. During normal differentiation, cortex and medulla stand in inverse relationship with respect to their state of differentiation. One component is seen to be developing, the other receding. In this process there is, so to speak, a struggle for survival, in which one component gradually effects the suppression of the other through the agency of a specific sex-differen-

tiating substance. This is the familiar "cortico-medullary antagonism" theory of Witschi (1932); and it appears to fit very well with experimental data from a variety of sources. The mechanism apparently is direct inhibition (Fig. 2).

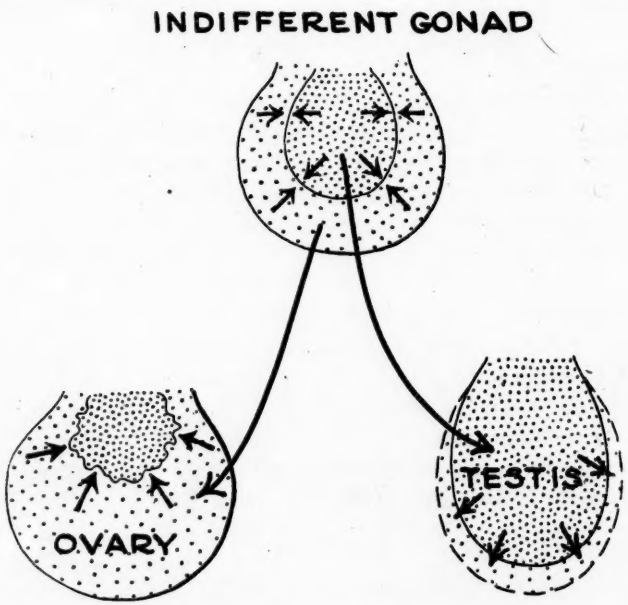
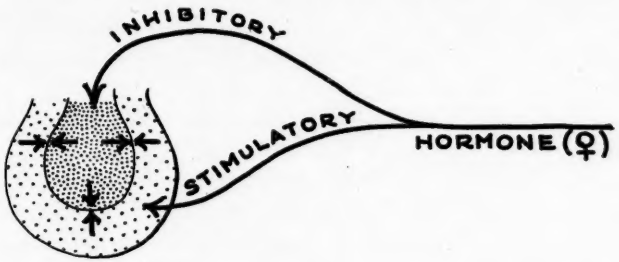


FIG. 2. Scheme illustrating "cortico-medullary antagonism" and the actions of inductor substances in early differentiation of the gonad (after Witschi).

Let us next look at the possible effects of introducing hormones from without on this mechanism, as illustrated in Fig. 3. In the freemartin the first effect of the infusing male hormone on the embryonic ovary is said to be a suppression of cortical growth (Lillie, 1917), regarded as equivalent to castration. This appears to be a direct



POSSIBLE ACTIONS OF EXTRANEOUS HORMONES ON GONAD DIFFERENTIATION

FIG. 3. Scheme illustrating possible modes of action of a hormone from without on the differentiating gonad components.

action by the incoming hormone, of an inhibitory nature, on the cortical component of the gonad, freeing the ovarian medulla from restraint; and its subsequent development is that of a sterile testis. Presumably the infusing hormone reinforces local medullary action. However, the evidence does not exclude a positive effect on the medulla by the male hormone.

In parabiosis and embryonic grafting in salamanders, the effects observed in gonads undergoing reversal appear histologically as cortical retrogression accompanied by medullary development, or the reverse; and in each case the component suppressed is heterotypic with respect to the type of hormone presumed to be working. While it is difficult to say in every case that inhibition definitely precedes activation, the circumstances are usually favorable to this interpretation; particularly when primary differentiation (in accordance with genotype) is followed by delayed reversal. In a reversing ovary large cortical growths undergo rapid reduction, apparently by direct action of the incoming hormone; for medullary development alone does not appear adequate to induce the effect. Conversely, in well-developed testes (chiefly in heteroplastic combinations) striking disorganization may appear in the medulla before cortical growth is sufficient to account for the result (Burns, 1935). Moreover, when

reversal does not go far, cortex may be completely inhibited with but little medullary growth, the "freemartin effect" in amphibians.

Directly supporting this view are the experiments of Harms, Ponse and others (Ponse, 1924), in which merely removing the testis in the adult toad permits the organ of Bidder, a persistent ovarial component, to develop into an ovary. Consequently, in amphibians it is generally believed that reversal of sex type in the gonad through the influence of hormones experimentally introduced is due to inhibition of the heterotypic gonad component, releasing the homotypic element for further development.

Here we may introduce evidence that the process of sex reversal utilizes the mechanism of normal development. In the differentiation of amphibian gonads, cortex and medulla are represented morphologically in accordance with the type of differentiation followed by the rete elements. That is to say, the differentiation of the rete apparatus determines the relative proportions of cortex and medulla. In reversal, infusing hormones exert a specific effect on the rete apparatus, inducing a change in sex type. This eventually reverses the quantitative relations of cortex and medulla, and the change may occur very early.

If the direction of differentiation can be reversed in this way by hormones from without, we are forced to look into the rôle of hormones and the postulated inductor substances in differentiation with respect to their autonomy. It is difficult to ignore the probability that we are dealing with identical agents, or at least substances so nearly identical that the biological results are the same. The argument may be tersely stated as follows:

(1) The inductor mechanism, with its specific inductor substances and its principle of cortico-medullary antagonism, is a system to account for early differentiation within the gonad.

(2) Embryonic hormones introduced from without can

also modify or completely reverse differentiation during the phase when inductors are normally presumed to act.

(3) The mode of action of hormones in reversal is through the mechanisms proper to normal differentiation.

(4) Since embryonic hormones can exert effects similar in every way to those ascribed to inductor substances, it seems likely that we are dealing with *different manners of distribution* rather than different agents.

During early development the sex-differentiating substances, produced locally, exert their effects locally, as do embryonic inductors in general. Later the substances appear in the blood, exerting their effects at a distance. This interpretation is supported by the effects of natural and synthetic hormones of adult origin on sex differentiation in the chick. We must, however, admit that in some forms the agents of primary differentiation can not be shown to act beyond the gonads, and in these forms hormones are apparently lacking. The toad is an example.

In the chick we now have much new evidence resulting from introduction of pure hormones into the incubating egg (Willier, Gallagher and Koch, 1935, 1937; Wolff and Ginglinger, 1935; and others). The preparations used have proved to be definitely sex specific (with exceptions to be noted); and since dosage can be regulated, one may study different degrees of response within the same time range. In higher dosages the heterotypic component of the gonad may be made to disappear entirely, while its counterpart reaches full expression. Complete reversal of sex occurs, making due allowance for the peculiar lateral asymmetry in bird gonads. Thus, according to dosage, the left testis readily becomes an ovotestis or a practically normal ovary, due to the presence of a potential cortical component (the germinal epithelium) possessing marked proliferative ability. The right testis reverses less completely, due to a lesser representation of cortex, and its testicular character accordingly is never entirely lost. The embryonic ovaries, which possess a more marked asymmetry than the testes, react more diversely

to male hormones. The rudimentary right ovary, which entirely lacks a cortical component, develops into a morphological testis with moderate doses; but strong treatment is required to produce a testis from a left ovary, and actually the cortex is never entirely inhibited. These effects are definitely proportional to dosage.

A peculiar exception to the general rule must be noted. The male urinary hormone androsterone and its derivatives, in addition to the masculinizing effects mentioned, induce the development of a slight cortex on the left testis of male embryos. We shall return to this later.

Although a specific effect of the injected hormones is established, there is again the matter of how the action is exercised. According to Willier and his colleagues when either hormone is administered to an individual of opposite sex, the first observable effect is actually an increase in the size of the homotypic component. This indicates direct stimulation; but is it possible to say there has not been, as yet, any inhibition of the heterotypic tissue? Mere absence of histological degeneration does not prove this point. Somewhat later, evidences of retrogression in the heterotypic component appear. The effect may be due to direct activation of the homotypic component by the infusing hormone or to depression of the heterotypic component; and a final conclusion is not possible.

However, in this connection we may mention the results of castration in the female chick soon after hatching (Domm, 1927; and others). This experiment is exactly comparable to castration of the male toad. Removal of the dominant left ovary allows the rudimentary right gonad to resume its development. Since in this structure the cortical component is almost or quite lacking, it develops as a testis; clearly the abolition of an inhibitory influence exercised by the functional ovary. It appears that the mechanism is like that in amphibians.

Up to the present we have too little evidence to decide this problem in mammalian gonads. The early disappearance of the cortical component of the freemartin ovary indicates the same mechanism.

The Action of Hormones on the Gonoducts

The mode of action of sex hormones on the duct systems is also a debatable question. In larval and immature amphibians the gonoducts long remain in a relatively unmodified condition. During this time development seems to be independent of hormones. In females the wolffian ducts remain as the mesonephric ureters. In the males of many species well-formed, but unspecialized, mullerian ducts persist throughout life. After castration the ducts, in both sexes, return to a condition approaching the unspecialized state, indicating that these structures should be readily amenable to reversal; and such indeed is the case. Conversion of rudimentary male oviducts to a state approaching or equalling the functional female structure has been demonstrated by Ponse (1924), Welti (1925), Adams (1930) and others. Transformation of the female wolffian duct into a vas deferens is less perfect, but marked hypertrophy of the duct and associated tubules of the "sexual kidney" has been secured (Ichikawa, 1937). In each case castration was followed by implantation of a gonad of opposite sex or by the spontaneous development of the organ of Bidder into an ovary. These effects must be due to *direct stimulation,* since they are superimposed on the castrate condition. Witschi (1936) and many others have secured similar results in late development when functional gonads of both sexes reside in the same animal.

Introduction of pure hormones into the chick embryo is followed by specific effects on the gonoducts. Female hormones in genetic males in some manner suppress wolffian ducts and stimulate development of mullerian ducts, sometimes enormously. Are these effects due directly to the experimental hormone or to the change of status in the reversing gonads? For example, when female hormones are working in genetic males, the growth of the male mullerian ducts may be ascribed to (a) direct stimulation or (b) release from inhibition exerted by the medullary component of the testis. Also a certain amount

of cortical growth may appear on reversing testes before the oviducts begin to respond; and here is a third possible source of the stimulus. Perhaps the lag in the response of the mullerian duct means nothing more than a somewhat higher threshold for this structure. A clue may lie in the effects of female hormones in genetic females. The ovaries themselves show no increase in size or other evidence of stimulation, but the mullerian ducts are greatly

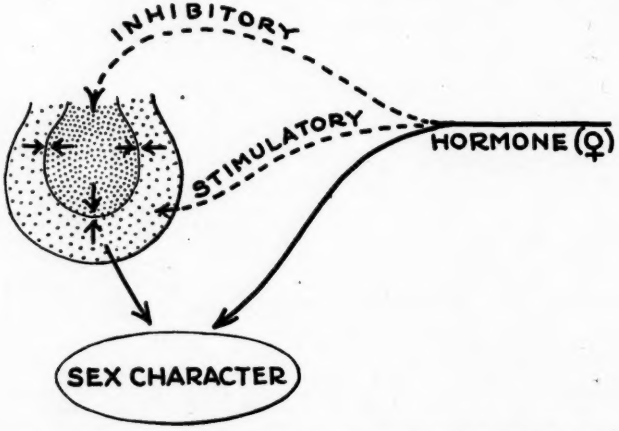


FIG. 4. Scheme illustrating possible modes of action of a hormone from without on a developing sex character. The hormone may act (a) directly on the character in question; or through the mediation of the gonad. In the latter event, the effect may come (b) as a release from the influence of the heterotypic element of the gonad; or (c) as direct activation by the homotypic element. The effects (a) and (c) may be additive.

hypertrophied. While the evidence is inconclusive, on the whole the theory of direct stimulation of the mullerian ducts is more probable and is in accord with the facts in amphibia. Female hormones have no effect direct or otherwise on the male duct system.

When the testis hormone acts in genetic females (Willier, 1937b) corresponding results follow. The wolf-

fian ducts are strongly stimulated; oviducts are always inhibited, so strongly in some cases as to indicate a direct action. The testis hormone, therefore, behaves in all respects as a male hormone should. Male hormones of urinary origin, however, have an added effect at once so unconventional and so definite as to require special consideration.

Workers in the physiology of reproduction have often reported an estrogenic activity of the urinary male hormones. In effect, they behave both as estrogens and androgens, a matter into which we can not go here. In describing the effects of these substances on the gonads the induction of a small cortical growth on the left testis of genetic males was noted as an important exception. Now appears a marked feminizing action on mullerian ducts in both sexes, not evoked by testis hormone. The effect becomes still more unconventional when we except from this action the left female mullerian duct (the future functional oviduct), which is actually suppressed. This fact remains a riddle unless we consider also the androgenic effect of the hormones. More active than its sisters the future functional oviduct may be especially susceptible to inhibition. We must take special account here of laterality, involving perhaps a difference of threshold (Lillie, 1931).

Reviewing these facts we may say: testis hormone produces reversal of the ovary with *suppression of embryonic oviducts;* androsterone also produces reversal of the ovary, but with *stimulation of oviducts.* In each case the effects on the gonad are the same; it is, therefore, possible to ascribe the behavior of the oviducts to direct inhibition by testosterone, and to direct stimulation by the androsterone group, through their special estrogenic property. The powerful stimulatory effect of testosterone on wolffian ducts already suggests direct action. These effects are definitely proportional to dosage.

In the freemartin, as we have seen, the ovarian cortex is destroyed very early—the "castration effect"—and

failure of the mullerian ducts to develop may be due either to this or to inhibition by the infusing male hormone. Conversely, differentiation of the male ducts may be due to positive stimulation or to removal of the inhibiting influence of ovarian development. But if the castration effect, which is complete even in the lowest grade of freemartin, is to blame, it is difficult to account for the wide variation of the ducts in subsequent development. The question is, then, whether the effects are due to the infusing hormone or to one arising in the freemartin testis. In either case a hormone of male type stimulates one duct while inhibiting the other.

Recently we have had reports of a real freemartin effect produced in the rat fetus by synthetic male hormones—compounds of testosterone (Greene and Ivy, 1937). In the chick testosterone acts as a true male hormone. Given to pregnant rats at different stages of gestation, female offspring exhibit graded modifications of the duct systems correlated with the stage at which the hormone was administered. The wolffian ducts are progressively modified, finally approaching the normal male condition. Mullerian ducts, inversely, are suppressed. Male offspring are not modified, nor does a hormone of female type (estradiol) modify males; of which more later.

While no final conclusion can be drawn concerning the action of hormones on the gonoducts, we receive this distinct impression. The true male principle acts directly on both ducts, stimulating wolffian ducts and inhibiting mullerian ducts. Female hormone when present acts positively on the female duct system, but is probably without effect on the differentiation of male ducts.

THE EXTERNAL GENITALIA

The external genitalia in mammals show a wide diversity, based, however, on variations of a common plan. As we have seen, they develop in both sexes from common rudiments. Their exceptional behavior in the freemartin is well known and it has been suggested that these parts

may be largely somatic in their mode of development or that their threshold to hormones is high. It is not possible to survey the large literature in this field. Let us as a matter of expediency consider the data referring to one form, the rat, thereby avoiding too great a diversity of problems.

The view that development of the external genitalia is largely somatic is untenable, for there is now much evidence against it. The results of Wiesner (1934) should be stated. In infant rats at birth there is slight sexual dimorphism. Castration is followed by cessation of development of the male genitalia. Females are unaffected. When castrates of either sex are given male hormone, there is approximation of the normal male condition, and normal females also respond in this manner. Administration of female hormone does not alter the castrate condition in either sex. Apparently male hormone alone conditions differentiation, and by direct action; female hormone is unessential. This is the basis of the mon-hormonic theory of Wiesner, who has extended this view to cover all development, supporting it by arguments from many sources. Notwithstanding the status of these structures as definitive female parts, from their hormone reactions they appear to be essentially male. Recently confirmatory evidence has come from a number of places. We may mention Dantchakoff (1937); Greene and Ivy (1937); and Hamilton (1937); the latter extending some of these findings to primates. However, none claim that female external genitalia are completely convertible to male, at least not under the conditions tested. There is always a certain deficiency of size or detail, especially in the incorporation of the urethral groove. What is emphasized is the importance of time in the determination of these parts. Their final character is to a degree imposed upon them very early, and perhaps within a very brief span. Lack of conditioning at the critical stage may be as positive in its after-effects as real conditioning. This makes the practical status of these structures as "non-alternative" parts a special difficulty in reversal.

One is impressed with the apparent unimportance of female hormone contrasted with the ability of male hormone to remodel female external parts. We have long been puzzled by the fact that in the fetus of mammals male development pursues its normal course untroubled by large quantities of female hormone in the placenta and amnionic fluid. We should seriously consider the possibility that in mammals a mon-hormonic system of control largely prevails, perhaps especially evolved as an adaptation to intrauterine development.

V. Quantitative Aspects of Hormone Action

It has been stated, in referring to the action of pure hormones in the chick, that the effects observed are proportional to dosage. This characteristic of hormone action is so clearly established now that it is hardly necessary to point out its obvious relation to our present quantitative concept of genetic sex determination. Heteroplastic combinations in amphibians have also shown that when the two members of a parabiotic pair or a host-graft relationship are very different in size, the effects are greater in degree, the incidence of complete reversal is increased, and male dominance is abolished. Closely interrelated with the quantity (or concentration) of the sex-differentiating substances, is the factor of time. Both the *time of onset* of the action and its *duration* affect the result in a quantitative way. The findings of Wolff and Ginglinger and of Greene and Ivy may be cited again. In each case the time of treatment enters directly into the degree of response. Unfortunately we can not discuss these interrelations adequately; but their rôles in the results we have discussed are often implicit if not stated.

VI. Identity of Hormones and Inductor Substances

The administration to chick embryos of hormones proper to adult mammals modifies the process of sex-differentiation in the gonad from its early stages onward.

Directly or indirectly differentiation of the gonoducts is also controlled. The effects of testosterone on fetal rats have been described; and recently, also, Uchida (1937) has shown that grafts of the adult testis clearly modify sex differentiation in larvae of the salamander Hynobius. Development of males is greatly accelerated, while many females become intersexual. Thus, we can not avoid the fact that in early development hormones of adult type may be biologically equivalent to the sex-differentiating substances of the embryo. The same situation was shown earlier with respect to inductor substances and embryonic hormones in amphibian differentiation. We must face the possibility that sex-differentiating substances are the same for all stages of development. Differences in the response of sex characters at different stages of development can not be taken as proof that different agents are responsible, unless we can show conclusively that the observed differences do not lie in a gradual change in status of the characters themselves as development proceeds. Time of appearance, differences of threshold, past conditioning may each or all affect the behavior of a particular character.

Nevertheless we must concede that we have no way of knowing whether artificially prepared hormones are, in the form injected, identical chemically with the substances acting. They may be modified biologically within the organism. This possibility may eventually explain why relatively large doses of "pure hormones" are often needed; and why one substance may sometimes have opposite specific effects, as in the case of male hormones of urinary origin.

VII. Summary and Conclusions

In closing we may be permitted to draw certain general conclusions, which of course are not to be regarded as final. They are not necessarily the conclusions of those who have contributed the data.

(1) The embryonic basis of sex in vertebrates consists

of a series of rudimentary parts common to both sexes, having a common basis in the sex genotype. These rudiments have different times of appearance, different morphological constitutions, different periods of differentiation; therefore different and changing reactions to hormones during development.

(2) In the differentiation of the gonad two embryonic components, cortex and medulla, having the value of ovary and testis, interact by a mechanism of mutual inhibition. In sex reversal the hormone acting from without works by taking control of this mechanism.

(3) The differentiation of the gonoducts appears to be under direct control of the proper gonad component. The effects of hormones from without may also be ascribed tentatively to direct stimulation of the appropriate duct. Direct inhibition of the mullerian duct by male hormone is also indicated.

(4) The rudimentary parts of the external genitalia of mammals appear to be largely, if not entirely, conditioned by male hormones, lending themselves to a mon-hormonic type of control. Their early development from non-alternative parts presents special difficulties in sex reversal.

(5) The effects produced by hormones are definitely proportional to dosage and to the inevitable time factor; but the constitution of the embryonic primordia also enters in a very precise way, especially illustrated in birds.

(6) On the basis of evidence now at hand we must consider the view that the sex-differentiating substances are probably the same for all stages of development. Differences in the reactions of characters at different periods of development may be due to change in the status of the character itself as a result of gradual conditioning.

LITERATURE CITED

References in this paper to articles appearing prior to 1932 may be found in the bibliographies appended to Chapters IV, V and VII, "Sex and Internal Secretions." Baltimore, 1932.

Burns, R. K., Jr.
1935. *Anat. Rec.*, 63: 101–129.
Dantchakoff, V.
1937. *Compt. Rend. Soc. Biol.*, 124: 516.
Greene, R. R., and Ivy, A. C.
1937. *Science*, n.s., 86: 200–201.
Hamilton, J. B.
1937. *Endocrinol.*, 21: 744–750.
Ichikawa, A.
1937. *Jour. Faculty Science, Hokkaido University.* Series vi, Vol. vi: 71–88.
Wiesner, B. P.
1934. *Jour. Obst. and Gyn. British Empire*, 41: 867–922, and 42: 8–78.
Uchida, T.
1937. *Jour. Faculty Science, Hokkaido University.* Series vi, Vol. vi: 35–55.
Willier, B. H., Gallagher, T. F., and Koch, F. C.
1935. *Jour. Biol. Chem.*, 109: 99.
1937. *Physiol. Zool.*, 10: 101–122.
Willier, B. H.
1937. *Science*, n.s., 86: 409.
Witschi, E.
1936. *Bull. Soc. de Biol. de Lettonie*, 5: 79–85.
Wolff, E. et Ginglinger, A.
1935. *Arch. d'Anat. d'Hist. et d'Emb.*, 22: 219–278.

INTERSEXUALITY AND DEVELOPMENT[1]

DR. RICHARD GOLDSCHMIDT
UNIVERSITY OF CALIFORNIA

WHEN I coined the terms intersexuality and intersex in 1915 I intended to meet the following situation: The sex-intergrades which had been produced by racial crossing in Lymantria and which had been called gynandromorphs turned out to be different from genuine gynanders. Boveri's work had made it clear that a gynander is a spatial mosaic in which areas of male and female nuclear composition, respectively, are found side by side, and Morgan had improved upon this notion by pointing directly to the sex chromosomes instead of to the nuclei. The sex-intergrades in Lymantria, however, did not exhibit such a simple mosaic of female and male parts but appeared to be, as a whole, something between the sexes, with all intergradations existing. It had been found some years earlier that these intergrades are produced as a result of an abnormal quantitative relation or balance between female and male sex determiners, a balance which is fixed at a point in between the two normal balances for maleness and femaleness, respectively. Thus the inference, contained in the term intersexuality, was that this plus-minus intermediate balance also controls an intermediate development as far as sex is concerned. But already during the first years of experimentation I had noticed in studying the genital armature of the intersexes that their structure is not more or less intermediate but that an orderly series exists connecting the condition of the two sexes, a series which suggested some hidden law. As early as 1913 I had discussed this problem with Spemann and had held out the hope that one day I might find a solution which would be of interest to the experimental embryologist. This solution was found soon after the intro-

[1] Read at a joint symposium on "Sex Differentiation" of the American Society of Zoologists and the Genetics Society of America, American Association for the Advancement of Science, Indianapolis, December 28, 1937.

duction of the term intersexuality, when it dawned upon me that the observed orderliness of intersexual transformation was produced by the fact that organs and parts of organs which differentiated last in normal development were the first to assume the characters of the opposite sex in intersexes and *vice versa*. This led to the conclusion that intersexes are not the result of a development between the sexual extremes. Intersexes, moreover, could be defined as individuals which begin development with their gametic sex and from a definite stage on, the turning point, end development with the opposite sex. The degree of admixture of characters of the opposite sex to the gametic sex, *i.e.*, the degree of intersexuality, is then a function of the position in time of the turning point. This was called the time law of intersexuality. The two extreme members of such an intersexual series are complete sex-reversal; and, as the gametic sex may be male or female, both a series of male intersexuality and of female intersexuality with different morphology will exist. Therefore we may also state that the different degrees of intersexuality represent different grades of completeness of sex reversal after the turning point, either from female to male or from male to female. As in the crosses which produce intersexuality in Lymantria always one sex is normal, the two series of male or female intersexuality are easily checked. All the morphological and embryological work done with these intersexes in Lymantria bore out the correctness of the definition. During the past years some doubt has been expressed whether the definition agrees with the facts. Of course nobody has doubted that in Lymantria female intersexes start development as females and male intersexes as males. But it has been claimed that, after the turning point, development is not that of the opposite sex but is an intermediate intersexual development. This is supposed to mean either an actual intermediate development or a kind of irregular mosaic formation. Furthermore, it was argued that the turning point does not occur at different times in development in the case of different grades of intersexuality. However, no explanation for the

orderly gradation of intersexual types was offered by the critics. These contentions have been raised by Kosminsky on the basis of certain facts which appeared in a perfectly atypical case of intersexuality. Both Kosminsky and later Baltzer have combed the facts relating to Lymantria to find inconsistencies, but the results were very meager. It is easy to show that their criticism does not fit the actual facts and that the time law of intersexuality stands firmly established in Lymantria.[2]

From the standpoint of physiology of development the problem now is to understand how sex-determining conditions, called male and female, influence developmental processes, whether consecutively or simultaneously. A discussion of this problem is not completely independent of the knowledge of the genetic basis of intersexuality, which is by no means uniform. It is furthermore necessary to know whether the phenotype that is called intersexuality and which is to be explained on an embryological basis is of a uniform appearance, which again is not the case.

The following genetic situations are met with in cases of intersexuality as related to different types of intersexuality. There is first the clear case of Lymantria, where intersexuality occurs within the normal diploid number of chromosomes and is produced by an abnormal balance of the potencies of female and male determiners, whatever this may mean in detail. All grades of intersexuality may be produced experimentally, including sex-reversal in both directions, and the phenotype is the consequence of the working of the time law. But in addition to these simple features another type of intersex occurs in which a mosaicism of the gynandromorphic type is added to intersexuality. A definite race of Lymantria (from Gifu, Japan) contains a gene which in the presence of the proper balance for female intersexuality causes these intersexes of all grades to exhibit features of mosaicism in all organs.

[2] A short discussion of this point is not possible. The reader is therefore referred to a special paper in which a full discussion is found and where also the complete literature is cited. (In press in *Genetica*.)

Simultaneously, a disturbance of the typical seriation of morphological changes in the intersexes is observed. No interpretation of this rare, so-called Gifu type of intersexes is known, though chromosomal eliminations are suspected. A comparable, again very rare, case known only from a few exceptional broods of Russian races (Kosminsky) is confined to male intersexes. Here two or three mendelizing genes (if my analysis of Kosminsky's data[3] is correct) cause males, even in the absence of abnormal genic balance, to develop into what looks very much like typical intersexual males but, again, with the addition of mosaic features and irregularities in seriation of sex-transformation in the different organs. The case is as unexplained as the Gifu type.

The second closely related type of intersexuality is found in the triploid intersexes which were first obtained by Standfuss in saturniid moths after a species back cross. They were further obtained in other moths either after species back crosses (Harrison, Meisenheimer) or after crossing parthenogenetic and bisexual strains (Seiler). They were found also in Drosophila among spontaneous triploids (Bridges). The genetic situation is clear in Drosophila. Unlike in Lymantria the genic balance is not disturbed by special features of the sex determiners (their potencies). The unbalance is, moreover, caused by a change of the actual number of these determiners when, in a triploid, two X-chromosomes are present, faced by three sets of autosomes containing opposite determiners from those in the X-chromosome. The embryological results of this unbalance are not expected to be as clear as in Lymantria. A triploid is in itself already unbalanced and has morphological peculiarities independent of sex. A triploid further has no gametic sex in regard to the sex-chromosome mechanism as a diploid Lymantria intersex has. There is *a priori* no reason why a triploid intersex should start development with one sex; one might expect, moreover, an intermediate development of some kind. But

[3] See footnote 2.

actually Bridges and Dobzhansky found that the morphology of these intersexes points out that they begin development as male and continue it as female after a turning point.

The genetic situation is less clear in the triploid intersexes of moths. Here, certainly, a simple abnormal number of X-chromosomes is not involved. In the Saturnia-intersexes the chromosome number seems to be varying and subtriploid (Goldschmidt and Pariser), but in Seiler's Solenobia intersexes it is exactly triploid. There is another disturbing feature. These intersexes are not of one type in one cross, as in Lymantria as a rule, but they are variable through all grades of intersexuality. In Drosophila, where the grades are also variable, it has been made probable (Dobzhansky) that modifying genes may be responsible. This might also be the case in moths, though the range of variation is extreme. It is remarkable that the same extraordinary variation is also found in the rare mosaic type of male intersexuality in Lymantria as mentioned above. Still more remarkable it is that the triploid intersexes of moths also incline strongly to mosaicism, so that I suspected them to be a combination of intersexuality and gynandromorphism. Just as is the case in Drosophila, no genetic sex can be expected to be found in these triploids. Nevertheless, they obviously follow the same developmental law as in Lymantria: they seem to begin development as females with subsequent reversal to maleness. I could demonstrate this for Meisenheimer's material of Oporabia, and Seiler found it at least for the sex organs of Solenobia intersexes, though he is a little sceptical regarding other organs.

Also in Drosophila there exists a very different type of intersexuality, occurring within the diploid chromosome number and caused by a single gene (with the addition of specific modifiers). This is the case described by Lebedeff in *Drosophila virilis*. According to Lebedeff, who permits me to quote from his unpublished work, these intersexes correspond in general to the female intersexes in

Lymantria, namely, being genetic females which begin differentiation as females up to a certain turning point. The special feature, however, is the behavior after the time of reversal. Some organs actually develop into the male direction, but others complete their normal female development. Simultaneously, the imaginal disks produce by budding the male parts, so that finally such organs are present as male and female organs, *i.e.*, in a hermaphrodite condition. Even the ovary follows in a part of the intersexes this strange type of development. Only in passing I mention a third type of sexual abnormality which has been termed intersexuality in *Drosophila simulans* (Sturtevant) and which is caused by a single gene. It seems, however, that here another phenomenon of inhibition of sexual differentiation in females is involved which is yet unexplained. The latter is unfortunately also true for what seems to be genetic intersexuality in Daphnids (Woltereck, Kuttner, Banta, De la Vaulx). There is no doubt that it is heritable. But it is unknown whether it is diploid or triploid. There is also no morphological or embryological analysis yet available which would permit drawing conclusions. Many degrees of intergrades are found which may be arranged in a series; but no law is yet visible, and it is not known whether the case belongs to the known types of intersexuality or not. The same negative result applies to the so-called intersexes of nematodes, lice and a few others.

With this group of zygotic intersexuality may be contrasted a group of intersexes which in a general way may be called hormonic. Two major types of these are known. The true hormonic intersexes are genetic females or males upon which at a definite time in development the action of the opposite hormones is forced (freemartin, injection of embryos with hormones, etc.). The beginning of this action may be called a turning point, after which actually differentiation proceeds, where possible in the type of the opposite sex. The other type of intersexes belonging to this group are the intersexes of Bonellia. Here at least

the majority of individuals have no actual genetic basis of sex determination.[4] They are indifferent first and become female if left alone. Under the influence of a secretion of the maternal proboscis (Baltzer) or of K and Mg ions (Herbst) the indifferent larva develops into a male. We might call the active substance a hormone. Baltzer found that larvae which were attached to the proboscis only a few hours instead of four days became intersexual. Herbst found similar cases after insufficient action of the masculinizing ions. One should assume that the development of such a type of intersexes should follow the same lines as in a freemartin type based on a gametic male, namely, first development under the influence of the male-determining "hormone" and afterwards continuation of development as a female. This might be compared with the time law in zygotic intersexuality. (It is in fact something else, as no gametic sex starts development in one direction, but an indifferent larva which would otherwise develop in a female direction is masculinized by the proboscis hormone.) Actually Baltzer had given first this explanation in his earlier work. Later, however, he abandoned this obvious interpretation and claimed that development of intersexes in this case proceeds as an intersexual development, *i.e.*, simultaneously female and male, the detailed result depending upon a number of embryological factors, *e.g.*, relative reactivity of different tissues to male- and female-determining substances. I have tried to show[5] that the exact data furnished by Baltzer and his students do not bear out his contentions but are in good agreement with the obvious interpretation given originally by Baltzer and which is closely related to the interpretations derived for the other best known cases of intersexuality.

From all this material we may try to derive a picture of the embryological processes concerned in the development of intersexes. In order to consider under a single heading

[4] See footnote 2.

[5] See footnote 2.

all the cases which have been analyzed thus far, we may adopt a suitable nomenclature of the decisive actions, without discussing its merits. We know from all the material in vertebrates that the agency producing intersexuality is a sex-hormone. We know from the Bonellia case that a substance is involved which might be replaced by definite ions, and we apply therefore the term hormone also to this case. In the cases of purely zygotic intersexuality we can not get around the conclusion that the genic balance which controls sexual differentiation acts through the production of some determining substances which we might also call hormones for the sake of unity. Formerly, when we had to assume that these determining substances are acting only within the cells in which they are produced, the hormonic conception met with difficulties. Meanwhile, however, the insects have also been shown to exhibit the inductive type of development to a certain degree (Seidel), further, to produce real hormones under the influence of genes (the molting hormones). The use of the term hormones for sexual determining stuff is therefore less irritating.

The embryological problem of intersexuality is then to find how the sex-determining hormones interact with such developmental processes which lead to sexual differences. Undoubtedly the developmental system concerned in the production of intersexuality has two phases: the inductive agency for which we use the term hormones, and the reacting tissues of the developing animal. The first question is, therefore, whether we are dealing with two inductive stuffs for the two sexes respectively or only with one. In order to explain the occurrence of the turning point I assumed originally the presence of both determining hormones, one of which was in excess in the pure sexes and slowly overtook the other in the intersexes. Dobzhansky later suggested that it suffices to assume one inductor substance controlling one of the sexes above a certain threshold, the other sex being developed if the hormone remains below the threshold. Under this assumption only the sex-

genes within the sex-chromosomes would control the production of the respective hormone; the so-called genes of the opposite sex (in Lymantria actually a cytoplasmic property) would control the value of the threshold. The cases in insects may be interpreted equally on both assumptions. In the Bonellia case we do not need more than one "hormone," forcing upon the otherwise always female development the male type of differentiation. In vertebrates we do not know anything about the primary sex-determining stuffs, produced by the sex-genes; but certainly the secondary determining substances, according to Witschi, are female or male determining, whether they are identical with the sex-hormones *sensu stricto* or not. It does not seem to be very relevant whether both described situations occur or not; from the standpoint of development there is not much difference whether two types of differentiation are initiated by two specific inductor stuffs or by different concentrations of one.

The second member of the system, the reacting tissue, is of greater importance. As I have repeatedly pointed out in earlier papers, and as also is emphasized by Baltzer in his recent discussion of the problem, a series of different possibilities must be reckoned with. There are first two major possibilities. The reacting embryonic tissue or organ anlage may or may not have an alternative reactivity. An alternative reactivity means that the same group of cells may be induced by the inductor stuff to develop either in female or male direction. The resulting sexual parts will then be called homologous. To give examples: Primordial sex-cells will develop into ovarial or testis tissue; a dorsal part of the last abdominal segment in moths will differentiate into the male hook-like uncus or the female ovipositor; the stomodaeum in Bonellia will develop into an œsophagus or a sperm tube. How do such organs of alternative differentiation behave in intersexuality? According to the turning point conception they will stop their direction of differentiation when the inductors of the other sex begin to act, and they will

continue development with the other sex. This is of course only possible if undifferentiated tissue is left which has still the capacity of changing its direction of organization. This type of development may be seen in a gonad: The primordial oogonia simply continue as spermatogonia and *vice versa;* but already differentiated egg and nurse cells as well as spermatids remain as they were[6] if they are not phagocyted. Corresponding cases are found in all sexually different organs of Lymantria. In some cases this is easy to demonstrate. If, however, simple quantitative differences are involved it is more difficult, because the visible result will be actually intermediate: a narrow cavity in one sex, which has begun to be formed but is forced to widen after sex-reversal towards the condition of the other sex, will reach an intermediate condition at the end of development.

We mentioned already that Baltzer and Kosminsky assume that after the turning point development is intermediate in insects and correspondingly in Bonellia after premature removal of the larva from the proboscis. This would mean either that both inductor substances act simultaneously with the result of a compromise development. Nothing of this kind can be demonstrated clearly in the known cases. Even in Bonellia the facts are not in favor of such a view.[7] In analyzing such facts one has to allow of course for simple mechanical impossibilities, *e.g.,* a small organ of one sex may grow out into or towards a larger one in the other sex but not *vice versa,* etc.

But there is still another possibility: When the inductors of the other sex begin to act, the anlage of the organ of the gametic sex has reached a stage of determination at which development can no longer be stopped. Full differentiation in the direction of the gametic sex will then continue. We might express this also by saying that the hormone which acts after the turning point has no inhibitory action upon such an organ, in addition to

[6] Spermatids may continue an abnormal differentiation.

[7] See footnote 2.

not acting on it in a positive sense. In such a case, a visible influence of the hormones of the opposite sex after sex-reversal in development is possible only if the original anlage was not strictly alternative in reaction but consisted from the beginning of two indifferent parts, of which each can react only with male or female inductor. Such is certainly the case in vertebrates with cortex and medulla, and with Muller's and Wolff's duct, prepared for reacting only upon the stimulus of one of the hormones. This situation would permit the stimulation of the anlage not corresponding to the gametic sex, without necessarily oppressing the other (though an inhibition is usually found), and both parts may therefore develop side by side into a more or less hermaphroditic condition. It seems that this situation, typical for vertebrates, may occur also in insects. I demonstrated that in the gonad of Lymantria probably a separation of two sexual anlagen, corresponding to cortex and medulla in vertebrates, exists. In Lymantria intersexes, however, always one is suppressed after the turning point. In triploid moths and in Lebedeff's case of *Drosophila virilis* the situation of the vertebrate type is found that the anlage of the non-genetic sex is stimulated after the turning point without inhibition of the parts prepared for the gametic sex. The result is a condition with both sex-organs present. These last examples show that in the production of certain types of intersexuality the sexual alternative of an anlage may be either of the completely alternative type: one group of cells may react upon the induction with male or female development; or it may be of a restricted alternative type: the organ anlage as a whole has the alternative reactivity, but from the beginning the organ has been subdivided into two parts, each with reactivity to only female or male inductor.

This condition may be considered to be a transition between the alternative reactivity of an anlage and the second possibility mentioned before, where a given group of embryonic cells has the potency to form only an organ of

one sex. (Of course one might also call this an alternative between something and nothing.) In this case a sex-character of one sex is without a homologon in the other sex. In case of intersexuality there are now two possibilities. The first is that the genetic sex has the organ in question. If it is formed before the turning point, it will either stop differentiation at the turning point. In the fully developed individual such a part would appear as a preserved embryonic condition of the organ; or, in the case of an organ of simple spatial arrangement (a process, a fold, a tube, a hook) the result would appear intermediate, without actually being this. Or the organ in question is not influenced by sex-reversal and therefore finishes its differentiation. Examples are known for both types of behavior. For the first, we mention the condition of the sperm tube in Bonellia intersexes which Baltzer misinterprets (as we believe) as produced by intersexual development. The second type may be illustrated by the segmental ring in the genital armature of male intersexes in Lymantria.

The second possibility is that the organ without homologon in the other sex is present not in the gametic sex but in the sex appearing after the turning point. The organ in question may appear then in the intersex, provided the cells in the proper locality of the embryo may still be able to differentiate at the time of the turning point. The result may then be either no differentiation (with too late a turning point) or complete differentiation or any stage in between according to the amount of differentiation still possible in the time left and in the neighborhood of the other organs. Beautiful examples for all these possibilities are found in the genital armature of Lymantria and the one extreme grade also in the female setae of Bonellia, normally developed in high-grade male intersexes (Baltzer's female intersexes).

There is one point regarding intersexual development in which all authors agree (but for the Bonellia case), namely, that sexual differentiation begins always with one

sex. In diploid intersexes this is always the genetic sex as given by the chromosome constitution, independent of what type of sex genes these chromosomes contain. (If the sex-genes alone are considered, the genetic sex of a zygote with abnormal balance of sex genes is always intersexual.) In triploid intersexes, however, no gametic sex in this sense exists; both, chromosomal and factorial basis, are intersexual. Nevertheless, development starts with one sex, namely, the heterogametic sex. This sex has, as has been known since the first work on Lymantria, the genes of its own sex outside the X-chromosomes. This means that apart from the sex-chromosome mechanism each individual would be determined for the heterogametic sex: each moth would be primarily of female, each Drosophila of male constitution. From this it follows that to explain intersexual development we actually need only one type of inductor, namely, the one corresponding to the homogametic sex. This again is in favor of an alternative sexual control of development, as opposed to a simultaneous intersexual one, advocated by Kosminsky and Baltzer.

At this point we ought to discuss briefly the notion of the turning point, which has been used throughout this paper. Originally this conception was rather rigid, as it was assumed that in insects all determinative processes occurred within the cells. Though it was not assumed that this switchover is actually occurring at exactly the same moment all over the body, it was recognized that certain local differences may exist which influence the reaction and the turning point appeared thus as an abstraction with not too much weight on "point" or, as Bridges expressed it, as an idealization of the actual condition. With our present knowledge of embryonic differentiation in insects the situation is somewhat more complicated. If an induction is involved, the time which this induction needs for becoming effective counts. And if the induction is started independently in different parts of the body, say in individual imaginal disks, another element of variability is added. Others will be taken into account, like readi-

ness of the tissue to react, different thresholds of reaction to the same concentration of the hormone and similar features, which Baltzer especially has pointed out. The turning point idea must therefore be applied sensibly in discussions of details and not with inflexible rigidity.

We have to ask finally whether any information is available regarding the type of action of the embryonic hormones of sexual differentiation involved in producing intersexuality. The answer is obvious where tissues are available which react only upon one type of sexual induction, like cortex, medulla, Wolff's and Muller's ducts in vertebrates. Here a stimulus of the type of ordinary hormonic action starts differentiation and, maybe, inhibits differentiation of the other anlage. Where, however, one and the same primordium has an alternative norm of reaction to one or the other stimulus (or maybe no stimulus and a positive one—the type found typically in insects), the problem arises how this stimulus is conducted to the primordium. Originally it was supposed that the inductor substance is produced simultaneously in all the cells of the primordium. Later work, however, made it probable that the induction—at least in organs developing from imaginal disks—is originated outside or at the base of the disk and flows over the disk after the fashion of a determination stream, in other words is of the type of an organizer action. (The individual disk then reacts as a whole.) This seems actually to be the case in moths and Drosophila. But occasionally mosaicism occurs within a disk which can be explained by special assumptions (see intersexual wing in Lymantria). There is one fact which actually seems to establish the parallel between inductor actions in Amphibia and in intersexual insects. The important parts of the male genital armature in Lymantria, valvae and penis are developed from an imaginal disk (Herold's organ). In certain male as well as female intersexes the valvae may appear in duplicate or triplicate. This has been explained as being due to the larger abdomen of the intersexes, which stretched the region from

which these parts grow out. In Amphibia recently, Holtfreter showed that a stretching of the organizer resulted in multiple inductions. Taking both cases together we conclude then that also in intersexual development of organs in insects an action of the inductor type enters the base of the imaginal disc.

It is obvious that a complete and detailed analysis of the facts regarding intersexuality in insects would require a much more intimate knowledge of Entwicklungsmechanik of these forms. But already at the present stage of knowledge the facts as revealed by the combined studies of genetics, morphology and development of intersexes are worthy of serious consideration by experimental embryologists.

LITERATURE CITED

Ancel, P.
1937. "Les hormones génitales et le développement sexuel embryonnaire chez les Vertébrés supérieurs." Dournées Médicales de Paris Internationales.

Full Bibliography up to 1931 in:
Goldschmidt, R.
1931. "Die Sexuellen Zwischenstufen," Berlin, J. Springer. pp. 1–528.

Full Bibliography up to 1937 in:
Baltzer, F.
1937. *Pubbl. Staz. Zool. Napoli*, 16.

More recently:
J. Seiler et coll., F. Baltzer
1937. *Rev. Suisse Zool.*, 44.
Goldschmidt, R.
Genetica (in press).
Lebedeff, G. A.
1937. Abstracts American Association for the Advancement of Science.

Vertebrates after 1931:
Witschi, E.
1934. *Biol. Rev.*, 9.
B. H. Willier et coll.
1937. *Physiol. Zool.*, 10.

BREEDING TO MEET ECONOMIC NEEDS[1]

METHODS APPLICABLE TO THE IMPROVEMENT OF LIVE STOCK

DR. H. D. GOODALE
MOUNT HOPE FARM, WILLIAMSTOWN, MASSACHUSETTS

Two questions ought to be asked about any method of breeding: (1) What rate of improvement can be made by its use? (2) How much improvement can be made?

Unless the answers to these two questions are known, a sound appraisal of the value of different methods of breeding is impossible. I propose, therefore, to discuss my topic from the standpoint of these two questions, but mainly from the first, since an answer to the second question is beyond our ability at present.

How shall rate of improvement be measured? Gregor Mendel demonstrated the importance of basing conclusions on a random sample of offspring. In live-stock breeding, then, the animals that are to become parents should be mated and the performance of all their offspring, taken as they come, recorded and, if necessary, averaged. But this simple and direct method of measuring rate of improvement is not in general use among live-stock breeders. Nevertheless, it is the only right way. Rate of improvement then may be defined as the average amount of change per generation made in the desired direction by all the offspring, taken as they come, of the parents of each generation. And since improvement by breeding—not improvement by purchase or gift—is under consideration, all later generations must be derived exclusively from the foundation stock. This statement is not a condemnation

[1] A symposium given before the Genetics Society of America at Indianapolis, Ind., the American Association for the Advancement of Science, December 30, 1937. A fourth paper entitled "A Barley Breeding Experiment," by H. V. Harlan, will be published elsewhere.

of any method of securing improvement in live stock but a clarification of the problem. Improvement in live stock can indeed be obtained by methods—methods of management, for example—which have nothing to do with breeding, but the problem before us requires that the improvement be made by changing inheritance. This can be done among other ways by discarding an inferior stock and replacing it with a superior stock, or the inferior may be raised to the level of the superior by "grading." These are perfectly good methods of breeding improved live stock. Nevertheless, since the time always comes in any breeding program when further improvement by the addition of superior inheritance from outside sources is no longer possible, the problem that can not be evaded is to find a method of breeding by which the best of the moment can be made better. The effectiveness of methods of breeding must therefore be subjected to experimental test. Yet in all the years in which mankind has been propagating animals very little has been done in a systematic manner to determine the relative merits of different methods of breeding and to learn which of the methods in use can be relied upon to effect improvement in inheritance.

The nature and mode of inheritance of those animal products—milk, eggs, wool, meat, and so on—which supply the world's economic needs, constitute an essential part of the problem. Almost without exception their inheritance is complex, for differences in productive ability do not fall into distinct classes, but pass by imperceptible degrees one into the other. Variation is therefore of the continuous kind and the breeder is compelled to work with inheritance, which can not be managed as easily as that of most characters used in genetic research. Moreover, he can not look to mutations to forward his work because, as Theodosius Dobzhansky says in his book, "Genetics and the Origin of Species," published late in 1937:

> The mutation process has not been brought under human control, since with or without X-rays one is unable either to obtain mutations in specified genes or to make the genes mutate in specified directions. Mutations remain haphazard.

Nevertheless, in contrast to this discouraging result, mankind has power to make large change in some characters at least—minute characters can be enlarged, variation can be developed where no variation of consequence existed, and the beginning of human control over processes which man has long sought to control but with no great success, may be at hand. One method of breeding at least has done these very things. Others may exist but they can only be found by search—a search that requires, among other things, knowledge of rate of change to be obtained by the use of a particular method or system of breeding.

Although little or no systematic study has been made of the effectiveness of various methods of breeding in promoting improvement in live stock, the results of experiments on what is called "selection" have been published, some of which can be used in measuring rate of improvement. The results of other experiments, such as those involving asexual reproduction which can not be applied to obligatory bisexual animals, are not suited to our purpose. The large volumes of published statistics, such as the records of the dairy breed associations, D.H.I.A., egg-laying contests, R.O.P. associations, race-track records, etc.—valuable in many ways—have such serious defects of one kind or another that they do not aid us in our search for superior methods of breeding. Indeed, I find myself in something of a predicament because the standard proposed for appraising methods excludes so much that little remains. Nevertheless, breeding methods can not be correctly appraised without adequate means of comparison. Moreover, as the man engaged in raising animals for their products is obliged to deal with all offspring as they are born, the standard fits commercial needs.

Strict application of the standard of appraisal would exclude from consideration most of the body of information relating to methods which in the present stage of knowledge—better, lack of knowledge—is not without its use. The plain truth is, there is no body of reliable facts on which to determine the relative value of various breed-

TABLE I

SUMMARY OF A NUMBER OF EFFORTS TO OBTAIN IMPROVEMENT BY VARIOUS METHODS AND IN VARIOUS FORMS BY SELECTIVE BREEDING

Experiment	Date	Material	Character	Method of Breeding		Change		Number, or Approximate Number of generations[1]
				As Stated	Inferred	Direction	Rate	
Castle	1908–18(?)	Rat	Extent of pigmentation	—[2]	Phenotype	+	.23 Grade[3]	20
						−	.14 "	21
Cole, *et als.*[4]	1913–20	Soy Beans[5]	Iodine number	Phenotype		+	0.0	7
						−	1.8	7
Cornell Agr. Exp. Station	1913–32	Poultry	Egg number	Complex[6]		+	5.2 eggs[7]	19
						−	0.0 " [8]	19
Hutcheson[4]	1901–13	Wheat	Neck length	Centgener		+	None	13
						−	None	11
Hyde	1924[11]	Drosophila	Proportion of adults from eggs	—	Phenotype	+	None[9]	4
						−	10%[10]	4
Illinois Agr. Exp. Station	1896 on	Corn	Per cent. Oil	Family (modified)		+	.18%[10]	40
						−	.10%[10]	40
	1896 on	Corn	Per cent. Protein	Family (modified)		+	.41%[10]	40
						−	.10%[10]	40
Johannsen[4]	1902–07	Beans	Weight	Phenotype (Within pure lines)		+	None	6
						−	None	6
Lambert	1926–31	Poultry	Resistance to fowl typhoid	Progeny Test		+	6.6%[10]	6
Lippincott	1927[11]	Poultry	Egg number	Grading		+	40 eggs	3
Lush	1907–16	Swine (Danish)	Daily gain in weight	Progeny Test		+	.013 pounds	23
Lutz	1911[11]	Drosophila	Abnormal venation	—	Phenotype	+	.06[12]	16
					"	−	.2[12]	8
Maine Agr. Exp. Station	1899–1907	Poultry	Egg number	Mass selection[13]		+	2.6 eggs decrease[14]	9
	1907–14	"	" "	Genotype		+	See Figure 10	—
Mass. Agr. Exp. Station	1912–21	Poultry	Egg number	Family		+	16 eggs	9
	1921 on	"	" "	"		+	2.5 eggs	15
	1931–32	"	" size	"		+	2.2 grams	2
Mount Hope Farm	1923–29 and 1936 on[15]	Poultry	Egg number	Family		+	7 eggs	7
	1929–35	"	Egg size	"		+	1.5 grams	6
	1931 on	Mice	Body weight	"		+	.5 grams	16
	1932 on	"	White on head	"		+	one grade	20
	1927 on	Dairy Cattle	Milk Fat	Index		+	{ 2275 pounds { 100 "	3

TABLE I—(*Continued*)

Experiment	Date	Material	Character	Method of Breeding		Direction	Rate	Number, or Approximate Number of generations[1]
				As Stated	Inferred			
Oregon Agr. Exp. Station	1908–17	Poultry						
		Barred Rocks	Egg number	—		+	13.3 eggs	10
		White Leghorns	" "	—		+	11.7 "	10
		Oregons	" "	—		+	11.0 "	10
Payne	1915–18	Drosophila	Bristle number	—	Family	+	.18 bristle[16]	17 } 38
						+	.04 " [16]	21 }
Pope[4]	1917–32	Barley	Internode and length of spike	Phenotype		+	None	15
						−	None	15
Roberts & Card	1925–33	Poultry	Resistance to pullorum	Part individual Part Progeny Test		+	None	7
Shull, East, *et als.*	Various since 1903	Corn	Yield	Self-fertilization, followed by crossing			3%[18]	7[18]
Tower	1896–1904	Leptinotarsa	Pigmentation	—	Phenotype	+	Rapid, then	11
						−	stopped	11
		"	Size	—	Phenotype	+	4.5 points[17]	10
						−	3.5 points	10
Tryon	1934[11]	Rat	Learning	—	Phenotype	+	6.5 errors	8
						−	5.6 "	8

EXPLANATION OF TABLE I.—This table is an attempt to summarize much of the work on selective breeding. It includes only work that could be prepared before the Sixth Annual Meeting (1937) of the Genetics Society. It is not possible to discuss these experiments in detail at this time but a few brief footnotes are necessary.

[1] The number of generations is not always stated.
[2] A dash indicates that no statement of method of breeding has been found.
[3] Zero is taken as the average of the foundation stock.
[4] Included for comparison, though not strictly applicable to live stock breeding because the plants were self-fertilized.
[5] The foundation plant was clearly a heterozygote.
[6] Selection was made for more than egg number, and males were added from outside sources from time to time.
[7] Rate of increase characterized by sudden increases between periods of slight or no increase.
[8] Although breeding for fewer eggs the average slowly increased for fifteen years. The gain amounted to 32 eggs. Since then it has declined. Is the decline due to changed methods of breeding?
[9] The proportion of adults declined, although selection was for increase.
[10] This is the average per cent. as the observations are on a percentage basis.
[11] Date of publication.
[12] Per cent. of population.
[13] Mothers laid 160 eggs or more. Sires came from dams laying 200 eggs or more.
[14] *I.e.*, opposite direction of selection.
[15] Breeding for increased number of eggs discontinued while breeding for egg size.
[16] Stringency of selection relaxed after the seventeenth generation.
[17] Rate of increase indicates the possibility of further increase had the experiment been continued.
[18] Counting six generations of self-fertilization followed by crossing and a maximum increase in yield of twenty per cent., the average for the seven generations is three per cent.

ing methods. So, since adequate information is not at hand, use must be made of such information as is available. This policy has the merit of providing a general survey of much of the material available for study at the present time (Table I) whose principal value is to demonstrate the need for carefully conducted studies on the value of different methods of breeding. It should not be used to judge the value of the methods used in the several experiments listed in this table because this can not be done without fuller information. Moreover, as different methods of breeding have not yet been applied on a comparable basis to a single character in a single organism, we are in no position to praise or condemn any method. Nevertheless, we see that varying degrees of success have attended the efforts of those who seek improvement by breeding. Some have made no progress; others make good progress, which in some instances has not reached a limit.

Disregarding one or two possible exceptions, I know of no work planned with the idea of testing the effectiveness of methods of breeding in promoting improvement in farm animals except that which led to the establishment of the mouse colony at Mount Hope in 1930. We hoped, by comparing one method with another, to learn more about methods of breeding and possibly discover a method capable of making more rapid improvement than the family method upon which we rely. But as the work with mice developed, limitations imposed by time, space and effort led us to concentrate our efforts on determining the amount of change which might be made in suitable characters by this method. This was done because our work with poultry, beginning a full dozen years before the mouse colony was established, showed such steady improvement in those characters to which we were giving our attention that there seemed to be no end to the amount of improvement possible.

It is quite impossible to discuss the various experiments cited in Table I at length. Indeed, the lack of a common

denominator for rate of change prevents a fair comparison of the results of one experiment with another. So, certain topics are taken for further discussion: the family method of breeding, the possibility of quickly increasing the yield of dairy cattle by concerted action by dairymen, a comparison of certain efforts to improve egg yield, and a comparison of the per cent. of improvement in yield of corn resulting from inbreeding followed by crossing with per cent. of improvement by the family method.

The family system of breeding originated with Gregor Mendel, who appears to have been the first person to interpret the breeding qualities of individual parents in terms of the kind and proportion of children produced—all children, good, bad and indifferent being taken into account. The inclusion of all children differentiates Mendel's method from progeny tests—and they are by no means extinct—such as that of Varro some 2,000 years ago who, in statements cited by Lush in 1937, says: "The quality of a ram can usually be determined by his conformation and from his get." "You may judge them by their get if their lambs are of good quality." But why not always judge them by their get, whether good or poor, as Mendel judged the breeding quality of his peas?

In adopting the policy of interpreting parents' breeding ability in terms of the number and proportion of their offspring, Mendel rendered a very great service to mankind, because it made—together with other contributions—the modern science of genetics possible. But for some reason this service of Mendel's has not been appreciated as it should be, perhaps because other of his contributions have absorbed the attention of investigators. If this contribution of Mendel's were fully appreciated, it would be applied to all selective breeding—not merely to analytical experiments involving discontinuous characters. But, strange as it may seem, Mendel's method of judging parents by their offspring is not always taken at its face value in selective breeding experiments, even by men who have won distinction in genetics. Thus, Johannsen, writ-

ing on "Grad des Überführrungs vermögens," which may be translated "degree of transmitting ability" illustrates his point by two racing stallions mated to equivalent mares, one of which gets better offspring than the other. Nevertheless, he makes no use of this very excellent rule in his pure-line experiments so far as I have been able to discover, although his figures show that the average weight of beans from individual plants in the same pure line differ, one from the other. Again, failure to interpret parents by offspring may explain why winter egg production at the Maine Station jumped in one year—1907—from 20 eggs to 54 eggs and then remained unchanged for six years—the experiment apparently completed—because the desired goal of a pure line had been obtained in accordance with the pure-line concept and further progress was of course impossible! It is known now that an average winter production of 54 eggs does not represent the production of a pure line. Indeed, there is reason to think that the limit, if any, is much higher. Other experiments in selection in pure lines have attained the same end results as Johannsen's experiments, but in no instance that I have been able to find have parent plants been judged according to the character of their offspring. It is possible, then, that some one courageous enough to take the evidence at its face value, disregarding probable errors, may yet demonstrate that pure lines are not so unchangeable as they are supposed to be.[2]

Mendel worked with variations of the discontinuous kind, which permit ready classification of the offspring. But the principle of Mendel's method applies also to variations of the continuous kind. Parents can always be judged by the kind of offspring they produce, and from this judgment there is no escape. To make this judgment it is only necessary to examine the records of the offspring of each pair of parents by themselves. The family method of breeding requires, therefore, that each female be mated to a single male and that her offspring be recorded by

[2] See appendix.

themselves. Thus the offspring of one male and one female is taken as the unit of operation.[3]

The family method of breeding is progeny testing of a special kind. It seems possible to use it in more than one way, in so far as published accounts of its use are available. Of course, allowance must be made for peculiarities of reproduction in applying the family method to particular species of plants and animals. As very little has been written on its use, and as it is one of the best methods of producing continued change, if indeed it is is not the best, a statement of its use, as applied to mice and to poultry at Mount Hope, appears to be required.

There are three steps in its use. First: In each generation those untried individuals which are given an opportunity to become parents are taken from those families which as a whole show the most satisfactory amount of improvement. Second: These individuals are subjected to rigorous tests of their ability to promote further improvement because experience shows that when continuously varying characters are concerned, differences in ability to promote improvement exist among the children of a single female, no matter how good the family as a whole may be. It is, therefore, necessary to repeat the tests in each new generation if continued improvement is desired.[4] Third: Those parents proved good are, when necessary, re-mated to other parents proved good.[5]

[3] The family method might be called the sib method because the word "family" is also used in live-stock breeding to mean the mating of related individuals, or individuals related to some notable individual. It is important, then, to distinguish between these usages.

[4] For an early statement of progeny testing in improving a character of economic value see Pearl (1915).

[5] Footnote added as a result of discussion at Indianapolis on mass selection compared with the family method. In mass selection individuals are selected to become parents on the basis of their own phenotype and mated "best to best." The next generation is selected and mated in the same manner. The breeding ability of the individuals used as parents is never determined and so no use is made of this fact. In the family method, individuals are selected not only on the basis of their own phenotype but on the basis of the phenotypes of their full sibs in order to take advantage of differences in parental

The way the family method of breeding reveals differences in the breeding ability of parents is shown by a few examples of "family-sheets," as the records of individual families are known (Fig. 1, Tables II and III). The exhibit is necessarily confined to these few examples because when a family sheet is prepared for each female, and the breeding operations are on the scale required to promote appreciable progress, the family-sheets for any one experiment make a volume of considerable size. The information on these sheets is mostly used without modification or "correction."

TABLE II

FAMILY DIFFERENCES IN BODY WEIGHT IN GRAMS OF MICE AT TWO MONTHS OF AGE (CURRENT RECORDS). THE FAMILIES ON THE LEFT WERE CHOSEN TO ILLUSTRATE FAMILIES HAVING THE LARGER WEIGHTS; THOSE ON THE RIGHT THE SMALLER WEIGHTS. NOTE THAT OF THE TWO DAMS MATED TO SIRE 7008, DAM 8172 HAD LARGE CHILDREN WHILE 8166 HAD SMALL. IT IS CLEAR THAT 8172 TRANSMITS LARGE BODY WEIGHT BUT THAT SHE WAS MATED TO AN UNDESIRABLE MALE. AS SOON AS THIS WAS LEARNED SHE WAS TRANSFERRED TO A MALE THAT TRANSMITTED LARGE SIZE

Sire 6577 Dam 7717		Sire 7008 Dam 8172		Sire 7008 Dam 8166		Sire 6792 Dam 7872	
Sons	Daughters	Sons	Daughters	Sons	Daughters	Sons	Daughters
41.5	34.4	39.0	33.5	31.5	27.1	34.5	22.6
41.7	38.2	41.8		32.1	28.4	34.1	26.5
38.5	34.2	43.4		33.5	26.5	31.1	30.5
				32.2	28.5		28.2

Sire 6621 Dam 7852		Sire 7518 Dam 8869		Sire 7421 Dam 8648		Sire 7017 Dam 8364	
Sons	Daughters	Sons	Daughters	Sons	Daughters	Sons	Daughters
39.2	37.1	40.5	37.4	32.7	26.0	32.8	23.4
37.0	34.2	40.4	32.7	29.2	26.5	26.6	
41.5	35.1	41.0	28.7	28.7	25.7	26.5	
45.1	34.8		35.4		25.6		
42.5			31.5				

genotypes and to increase the chances of securing superior genotypes for testing. This is the first stage in the family method. In the second stage individuals of desirable genotypes are separated from those of undesirable genotypes by the character of their early progeny. In the third and last stage individuals of desirable genotypes are mated together in order to obtain more offspring in the direction of selection for further testing. Thus, the family method seeks to identify and use to the best advantage those individuals which demonstrate their ability to promote progress in the direction of selection.

TABLE III

THE DIFFERENCE BETWEEN TRAP-NEST PERFORMANCE AND BREEDING PERFORMANCE

Dam's Number	Dam's Egg Record	No. of Daughters	Daughters' Average Egg Record
	First Set		
E310	280	11	137
H632	280	11	220
G284	284	10	255
G709	286	11	167
G1430	282	11	201
H179	280	7	167
	Second Set		
H119	320	15	203
H352	300	6	154
H679	308	16	228
J376	302	6	249
J207	301	12	252
J938	313	6	184

The family method of breeding has been reduced to an established routine at Mount Hope Farm, which is sometimes called a breeding machine because of the fashion in which it sorts out and rejects or saves, as the case may be, individual parents for future use, and because of the steady improvement which results from its use. Experience with students demonstrates that this method of breeding is quickly mastered.

Several hours could be spent in discussing the family method of breeding, but since this is not possible, we turn now to the rate of change obtained when this method of breeding is applied to improving egg yield, egg size, body weight in mice and increase in size of white spot on mice (Figs. 2 and 3), and to the changes in distribution of the numerical values of the characters subjected to this method of breeding (Figs. 4, 5 and 6).

In addition to these demonstrations of the effectiveness of the family method of breeding for improvement, experiments in selective breeding have been made, one of which may have used the family system while the others certainly used a modified form of this method. The first is Payne's experiment in breeding for increased number of bristles in Drosophila where, as the flies were mated by pairs, it seems reasonable to suppose that the progeny of each pair were recorded separately and that the parents

A Jabot J1478 (G1430 × Handsome) × Jack J850 (H371 × Herald)

PEN NO. 10—KINGLAKE K1586

LADY of the LAKE **DAM NO.** L469

Kyffhauser K974 (J241 × Jo Pivet) × Kingfisher K6830 (H257 × Janitor)

Mount Hope Farm — Family Sheet

	Pullet No.	Hatched		Age at 1st Egg	Date of 1st Egg	Egg Weights 50th	Egg Weights 200th	Body Weights	Eggs to Oct. 1st	Oct.	Nov.	Dec.	Jan.	Feb.	Total	March April May	June July Aug.	Sept. Oct. Nov.	Year Total
Dam	L469	4/4/29	192½	177	9/28	58	57	3.50 3.50	2	19	19	20	22	19	101	72	68	21	258
	N 28	1931 3/26		187	9/29	51	56	4.00 —	1	26	24	25	23	21	120	54	58	14	245
	58	4/2		186	10/5	58	59	3.50 3.50		14	19	22	25	22	102	75	61	35	251
	59	4/2		169	9/18	56	63	3.60 3.40	6	23	22	16	19	19	105	66	54	17	234
	77	4/9		167	9/23	52		3.50 3.70	2	22	25	18	22	16	105	41	43	5	192
	109	4/9		189	10/15	57		4.00 4.15		8	23	18	21	16	85	Pr P D			
	127	4/16		191	10/24	56		3.50 4.00		6	13	24	24	12	79	38	23	10	150
	244	4/23		220	11/29	66		4.00 4.20			2	22	22	22	68	P D			
	182	4/23		201	11/10	61		4.00 4.00			15	22	20	18	75	59	P D		
	207	4/30		188	11/4	54		3.75 4.40			20	27	26	25	96	71	28	0	195
	208	4/30		168	10/15	55	62	3.75 4.00		12	24	23	22	21	102	66	55	29	250
	257	5/7		204	11/27	58		3.75 4.25			3	24	25	21	73	75	30	0	178
	258	5/7		200	11/23	56	58	3.75 4.25			5	23	24	23	75	72	55	20	222
	289	5/14		202	12/2	59	58	4.30 5.00				20	18	18	56	66	71	24	217
	Average	4/20		190	10/25	56	59	4.07		24	21	22	22	20	88	62	48	15	214

B Kilderkin K481 (J487 × Dr. Jekyll) × Kossuth K721 (J361 × Jeronimo)

PEN NO. 6B—LIMERICK L1707

KADAPA **DAM NO.** K591

FIG. 1. "Family sheets" used at Mount Hope in breeding for characters of economic value in poultry. The two families, A and B, illustrate some of the differences existing between families.

B Kilderkin K481 (J487 × Dr. Jekyll) × Kossuth K721 (J361 × Jeronimo)

PEN NO. 6B—LIMERICK L1707

KADAPA **DAM NO.** K591

Juvenal J796 (G1529 × General Grant) × Jesse J2648 (E782 × Haberdasher)

Mount Hope Farm — Family Sheet

	Pullet No.	Hatched		Age at 1st Egg	Date of 1st Egg	Egg Weights 50th	Egg Weights 200th	Body Weights	Eggs to Oct. 1st	Oct.	Nov.	Dec.	Jan.	Feb.	Total	March April May	June July Aug.	Sept. Oct. Nov.	Year Total
Dam	K591	3/29/28	121¼	163	9/8	53	55	3.50 3.45	19	23	23	13	22	22	122	79	71	50	277
	N 20	1931 3/26		178	9/20	57	—	4.65 4.50	6	13	19	16	3	18	75	61	57	8	200
	21	3/26		164	9/6	50	53	4.50 3.75	19	26	25	24	22	19	135	68	76	21	282
	22	3/26		154	8/27	48	56	3.90 3.25	30	28	24	23	26	21	152	63	71	32	283
	23	3/26		152	8/25	47	58	3.10 3.00	30	25	25	24	25	23	152	53	58	33	279
	51	4/2		146	8/26	48	53	4.00 3.50	22	25	25	22	23	23	140	67	63	28	268
	52	4/2		149	8/29	46	54	3.25 3.00	18	25	23	23	23	18	130	60	43	17	232
	53	4/2		157	9/6	48	58	3.50 3.35	13	26	25	23	22	23	132	52	36	2	220
	81	4/9		172	9/28	50	58	3.75 3.85	2	29	26	24	23	24	128	68	79	30	292
	82	4/9		182	10/8	51	52	4.40 4.30		18	23	21	20	18	100	61	72	30	255
	83	4/9		174	9/30	52	57	3.80 3.60	1	24	21	24	24	23	117	67	68	22	271
	122	4/16		154	9/17	54		4.25 3.90	6	16	14	16	15	13	80	41	38	22	163
	123	4/16		158	9/21	50	53	3.80 3.45	7	26	28	28	26	24	139	72	69	37	294
	124	4/16		161	9/24	49	55	4.00 3.70	4	29	27	27	25	21	133	53	63	35	267
	162	4/23		170	10/10	52	56	4.50 4.15		11	21	26	25	25	108	70	75	62	283
	163	4/23		168	10/8	50	55	4.00 4.15		19	25	23	23	23	113	70	75	40	283
	200	4/30		170	10/17	55		4.15 4.25		9	15	16	21	18	79	Pe D			
	251	5/7		191	11/14	52	59	3.80 4.00			13	27	29	26	95	68	60	50	266
	Average	4/10		165	9/21	51	56	3.96 3.75		24	23	23	22	21	118	62	63	29	257

FIG. 1.—*(Continued)*

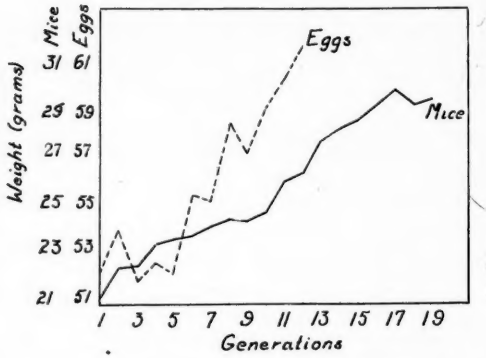


FIG. 2. Increase in body weight of mice (females) and of egg weight in fowls at Mount Hope under the family method of breeding. Breeding for increased body weight of mice began with the first generation but no efforts were made to increase egg weight until the sixth generation.

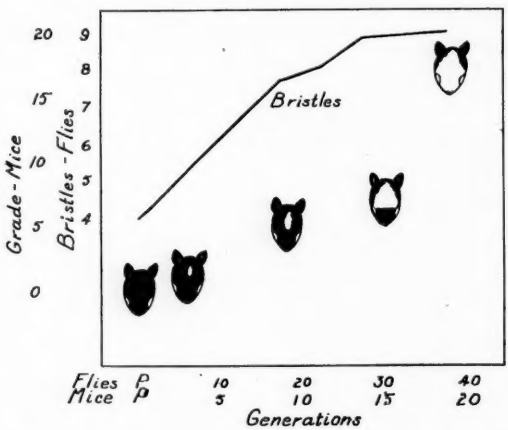


FIG. 3. Increase in number of scutellar bristles in *Drosophila melanogaster* (Payne) and in number of white hairs on the forehead of mice. The bristle number is averaged for each five generations except the first which indicates the bristle number of the foundation stock. The white spot originated in a single mouse having a few (12) white hairs on its forehead. It belonged to a self-colored race represented by the lower left-hand figure. Breeding for larger numbers of white hairs began with this one individual. The first appearance of four stages in the increase in number of white hairs (intermediate stages being omitted) is shown by the graph.

of the next generation, intentionally or unintentionally, were taken from families that showed change in the desired direction. The other experiments (Fig. 7)[6] are the classical Illinois experiments on changing the per cent. of oil and protein in the seed of maize by selective breeding, which are usually cited as examples of mass selection, but this is a mistake. Since the ear is the unit of selection

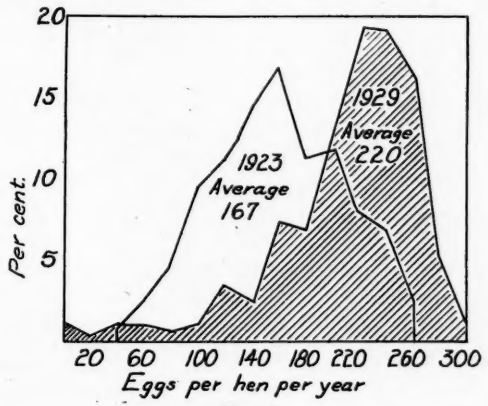


FIG. 4

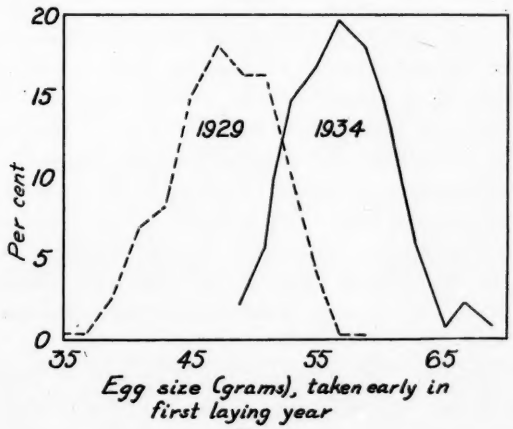


FIG. 5

[6] Dr. C. M. Woodworth and his associates of the Illinois Agricultural Experiment Station have supplied data and permitted its use in the graphs. My best thanks are due them for this assistance.

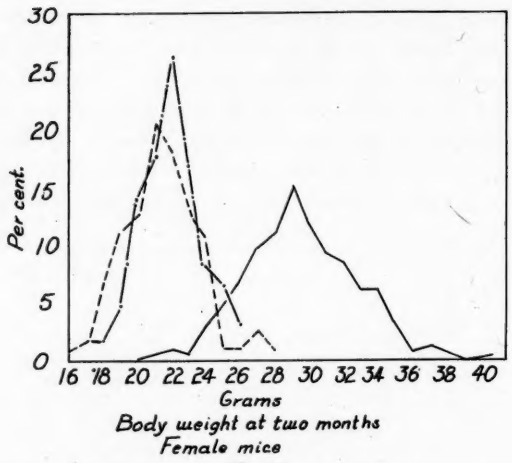


FIG. 6

FIGS. 4, 5 AND 6. Change in distribution of egg number, egg weight and body weight in mice (females) to show the shift in the direction of breeding. Figure 4, egg number. Figure 5, egg weight. Figure 6, body weight in mice (female) at two months of age. The distribution of weights early in the experiment is shown by the line of dashes - - - - ; the present status, by the continuous line ————. As a check, the distribution of the weight of unselected inbred agoutis is shown by a broken line ——— . ———.

each ear is a family having a common mother. As pollination is at random, the father of each family is the equivalent of one individual of average genetic constitution. Each mother plant is in effect tested against a common, and therefore uniform, male parent in each generation. From each ear a random sample of seeds is picked for chemical analysis. Seeds from ears (*i.e.*, families) showing the most modification in the desired direction become the parents of the next generations. This case is not then a case of mass selection but a special kind of family breeding.

The Illinois experiments with corn are of particular importance in connection with the improvement of dairy cattle by breeding, because owing to the small number of daughters born to each cow during her life-time, the family system as it is used with poultry or mice can not be

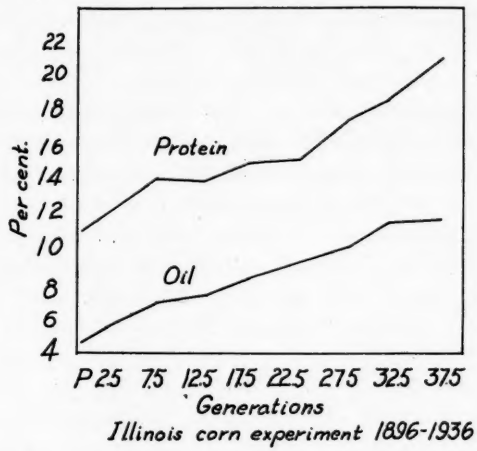


FIG. 7. Increase in per cent. of protein and oil in the grain of maize. Data from the Illinois Agricultural Experiment Station. Averaged by five-year periods except the first (P) which is taken as the average of the foundation stock.

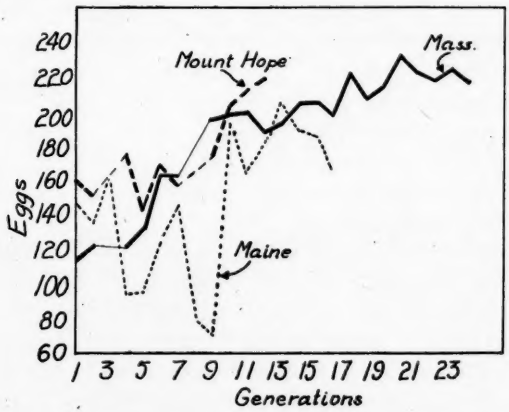


FIG. 8. Changes in Average Annual Egg Production at the Maine and at the Massachusetts Agricultural Experiment Stations and at Mount Hope. There are two periods at the Maine Station. During the first, dams were selected on the basis of their own records, 150 eggs or better, and sires on the basis of their dam's records, 200 eggs or more. During the second period breeding was on the basis of the pure line concept. The published averages are of winter eggs which are stated to be 27 per cent. of the annual records.

used for the improvement of dairy cattle. Bulls, however, can be tested in the same manner that the mother corn-plants are tested in the Illinois experiments. And as very large changes have been made in protein and oil content, it is evident that the same method should be successful in improving dairy qualities. That this is so is shown by our experience with it at Mount Hope, where it has been employed for more than ten years. We call it "index breeding" because a numerical value—the index—of the breeding ability of each sire is computed from the relation of daughters' productive ability to that of their dams.

Now Mount Hope has found that when one high index bull follows another in our herd, the average production rises steadily. It is no trick at all to transform a low-producing herd into a high-producing herd by index breeding.

TABLE IV

INCREASE IN MILK AND BUTTER-FAT YIELD BY USE OF INDEXED SIRES. THE NUMBER OF ANIMALS IS SMALL BECAUSE OF THE NATURE OF DAIRY CATTLE. THE RECORDS ARE CONVERTED TO A MATURE ADVANCED REGISTER BASIS

Generation	Number	Average Mature Equivalent Advanced Register Records	
		Milk	Butter Fat
P	38	11,876	583
F_1	32	15,757	757
F_2	6	18,700	884

From our experience with index breeding we are convinced that the milk yield of all dairy cattle of the country could be raised in like proportion within a few years, if it were not for human indifference and indolence. Dairy farmers are obliged to maintain some kind of bull in order to keep their cows in milk. They are obliged to feed and

They have been converted to annual records on this basis in order to make them comparable with the Massachusetts Agricultural Experiment Station and Mount Hope averages. At the Massachusetts Agricultural Experiment Station after an initial orientation period breeding was for increased number alone. After 1921 other characters were added. At Mount Hope, after the initial stage, breeding was for increased number alone through 1929, after which breeding was for increased egg size, egg number being maintained as well. Thin portions of lines indicate absence of one or more averages.

to milk them. By the addition of perhaps one per cent. to the labor they are now obliged to perform it would be possible to test all the several million bulls in service through this country and to preserve those that have high indexes. Moreover, there is no reason why every farm should not have access to the services of sires that transmit high production because progress in the use of artificial insemination is proceeding at so rapid a pace that good bulls can be made to cover every cow in America. If farmers would, the same rate of improvement that Mount Hope has attained could be made generally. Moreover, the scale on which bulls might be tested is so large that levels of production exceeding anything now known may be attained in due time.

Breeding to improve egg-laying ability has received much attention. The rate of improvement, measured by increase in the average number of eggs, has been published in seven instances, but a comparison of these averages would be unsatisfactory even if accompanied by detailed statements covering all their vagaries, partly because no uniform method of computing averages is used, partly because breeding for increased laying ability alone is likely to show a higher rate of improvement than when it is combined with breeding for improvement in other characters, and partly because of uncertainties as regards the method of breeding actually used. The plain fact is that if the relative value of different methods of breeding for improvement of live stock is to be determined, the experiments must be planned to this end. Some form of statement of results must be devised which permits of a fair comparison of the results of one method with another. However, a graphic comparison of the two phases of the work at the Maine Agricultural Experiment Station with that at the Massachusetts Agricultural Experiment Station[7] and at Mount Hope—both of which use the family

[7] The figures for average annual egg production at the Massachusetts Agricultural Experiment Station are available through the courtesy of Dr. Frank A. Hays. My best thanks are due him and his associates for permission to use these figures.

method of breeding—demonstrates the effectiveness of this method of promoting a constantly rising average (Fig. 8).

Close inbreeding for six or seven generations—self-fertilization—followed by crossing is a favorable method of increasing yield of maize and is applicable to live stock improvement by substituting brother-to-sister matings for self-fertilization. I am told by those most familiar with this method that the maximum gain in yield is about 20 per cent. over the yields of the most productive varieties of maize. We have applied the method to poultry, but the results are not encouraging. It is difficult to continue brother-to-sister matings and the offspring of crosses between inbred lines have made no conspicuous gain in egg production over the foundation stock. By contrast, the family method increased egg yield at Mount Hope over 30 per cent. during seven years.[8] At the Massachusetts Agricultural Experiment Station the increase by the same method over a much longer period—23 years—has been over 100 per cent. In mice, the gain in weight—which is a form of yield comparable to pork yields—is better than 40 per cent. in eighteen generations. The Illinois corn experiments have increased the percentage of protein and oil more than 100 per cent. in forty generations. It seems quite possible, then, that when the family method of breeding is applied to increasing the average yield of maize, a greater gain may be secured than is possible in the most favorable instances of self-fertilization for six or seven generations followed by crossing of the inbred lines.

A number of matters, such as the scale on which the breeding operations are conducted, the percentage distribution of family averages, breeding for uniformity of

[8] In stating the results of our work at Mount Hope, in breeding for increased egg yield, the average is the average of all the offspring of each new generation of males and females used as breeders. But the mouse averages include all offspring taken serially. The first method relieves the averages of weighting due to retention of parents successful in transmitting desirable qualities and to discarding the failures. But over a long series of generations the influence of this factor is so well distributed that it can be disregarded.

product where such uniformity is desirable, breeding for general all-round excellence, and so on, all bearing in one way or another upon the value of various methods of breeding, must be passed by. However, there are two points which I should like to discuss briefly in bringing this paper to a close.

First: The mating and reproduction of live stock is always in operation. It would seem that means might be found whereby a part of all this work could be utilized in transforming the art of breeding from the haphazard affair of to-day into a system of orderly information which would in due course separate good methods from poor, and place breeding on a solid foundation of knowledge. Perhaps the place to begin is with dairy cattle, where, in spite of the claims of the breed associations, there is no evidence that the improvement in Advanced Register averages is due to anything but improvements in feeding and in the selection of animals for test. But whether a beginning is made with dairy cattle or with other animals, it seems a great pity that more use can not be made of the breeding operations always under way to increase our knowledge of the effectiveness of different methods of breeding to promote improvement in hereditary qualities of live stock of economic value.

The second point aims also to advance knowledge of breeding and the promotion of improvement in the hereditary qualities of live stock. The indifference and indolence of the human race does not offer much encouragement that anything will be done about the first point, but mice and other laboratory animals can be made to serve the purpose, to some degree at any rate. It is perfectly possible by the use of these animals in studying breeding methods to advance our knowledge not only of breeding but of the processes of inheritance. We may even hope that these efforts will aid in revealing the secret of the method of the origin of species, and the evolution of more complex forms of life from less complex forms. The crossing of diverse races and forms has enlarged our

knowledge of the process of inheritance to a degree which, when viewed from the beginning of the century, is incredible. But crossing of races is not the normal method of reproduction nor the only possible method of research. The normal method in nature is free reproduction between neighboring individuals. While this type of mating is not the family method, perhaps the family method is not without influence, for if, of several progenies, a larger proportion of some survive to reproduce than others, then something of the family method exists in nature and could be effective in producing change over long periods of time. A comparative study of the value of different methods of breeding in effecting change may therefore prove to be as valuable a means of research into the problems of evolution as the methods now in use.

APPENDIX

I

Having criticized the technique of selection experiments in pure lines the application of the family method of breeding to this case should be illustrated by a concrete example.

The test should begin with a random sample of the seed from a single plant of a species or variety such as beans or wheat whose normal mode of reproduction is self-fertilization, say 30 seeds. These are weighed individually and planted. The next generation, F_2, is produced by planting all 30 seeds. The seed of each plant is harvested by itself, the seeds weighed individually and the average computed for each mother plant. The process is repeated for F_3 and F_4, the number of families increasing to 900 in F_3 and 2,700 in F_4. Selection may now begin, say in two directions—one for heavier seeds, the other for lighter seeds. The 30 F_4 families having the highest average weight are taken as the beginning of one line, the 30 F_4 families having the lightest seed are taken as the beginning of the other. All the seed from each family is planted, making a total of 1,800 F_5 plants, 900 in one line and 900 in the other. In each of these the 30 families having respectively the highest and the lowest average are used to produce F_6. This process is to be repeated generation after generation. Of course care is taken to see that environmental factors do not ruin the experiment.

II

Since a small part only of the many methods of breeding were mentioned in the body of this paper, the omission is supplied by the following list. Of course, all breeding except possibly random mating involves some kind, degree or method of selecting parents. All these methods or plans are in use either singly or in combination.

Methods of Breeding

On basis of Relationship

Brother to sister
Brother to sister, followed by crosses
Relatives, less close than brother to sister, such as cousins
Ancestors[1]
Line breeding

On basis of Phenotype

Phenotype alone
Correlations between phenotypes[2]
Balanced breeding[3]

On basis of Genotype

Preliminary selection on one or more of several bases; final selection on basis of progeny
Family method, see text
Hagedoorn's pen system for cocks

On basis of Objective

Breeding for uniformity
Breeding for a single character
Breeding for several characters at one time

Miscellaneous

Grading
Random matings
Flock matings
Pen matings
Individual matings
Inter-racial or interspecific crosses
Blood lines
Pedigree[4]
Crossing followed by selective breeding
Owner's reputation; *e.g.*, Mr. Jones or Mr. Smith[5]

[1] Breeding by ancestors means the purchase and use of an animal, especially a sire, because of one or more famous ancestors.

[2] Breeding by correlation between phenotypes is breeding for one thing to get another with which it is believed to be correlated, such as breeding for show type to get milk.

[3] Balanced breeding mates animals with defects to one without these defects or with a defect in the opposite direction. Much used by fanciers but applicable to economic characters.

[4] Pedigree breeding requires satisfactory knowledge of the pedigrees. It involves much more than the ancestor method.

[5] Breeding by reputation is an attempt to improve one's stock by purchase from a breeder who has gained a reputation for having the kind of stock one wants.

Names: *e.g.*, Dairy Cattle—May Rose, Pontiac, etc.[6]

"A maximum amount of pleasure and profit will reward the efforts of those who build on the Maxims." Advertisement, page 1150, *Guernsey Breeder's Journal*, Vol. 52, December 1, 1937.

Breeding from all but the poorest

LITERATURE CITED

Castle, W. E., and J. C. Phillips
1914. Pub. Carnegie Inst., No. 195.

Cole, L. J., E. W. Lindstrom and C. M. Woodworth
1927. *Jour. Agr. Res.*, Vol. 35.

Dobzhansky, Theodosius.
1937. "Genetics and the Origin of Species," Columbia University Press, New York.

Dryden, J.
1921. Oregon Agr. Exp. Sta., *Bul.* 180.

Goodale, H. D.
1922. Mass. Agr. Exp. Sta., *Bul.* 211.
1937. AMER. NAT., Vol. 71.
1938. *Jour. Hered.*, Vol. 29.

Hall, G. O.
1935. *Poultry Sci.*, Vol. XIV.

Hays, F. A.
Mass Agr. Exp. Sta.—Letter.
1914. AMER. NAT., Vol. 48.

Hyde, R. R.
1924. *Jour. Exp. Zool.*, Vol. 40.

Johannsen, W.
1909. "Elemente der Exakten Erblichkeitslehre," Jena.

Lambert, W. V.
1932. *Jour. Immunol.*, Vol. 23.

Lippincott, W. A.
1927. "Poultry Production," fourth edition, Lea and Febiger, Philadelphia.

Lush, J. L.
1936. Agr. Exp. Sta., Iowa State College, Agr. Mech. Arts, Res., *Bul.* 204.
1937. "Animal Breeding Plans," Collegiate Press Inc., Ames, Iowa.

Lutz, F. E.
1911. Pub. Carnegie Inst., No. 143.

Mount Hope Farm
1931. "Prepotent Cockerels," printed privately.
1935. "Early Egg Size," printed privately.

Payne, F.
1918. Indiana University Studies, Vol. 5, No. 36.

[6] "We congratulate ourselves because at the foundation of our breeding program will be found *six Maxims.*"

Pearl, R.
1915. AMER. NAT., Vol. 49.
Pope, M. N.
1935. *Jour. Am. Soc. Agron.*, Vol. 27.
Roberts, E., and L. E. Card.
1935. Univ. Illinois, Agr. Exp. Sta., *Bul.* 419.
Smith, L. H.
1908. Illinois Agr. Exp. Sta., *Bul.* 128.
Tower, W. L.
1906. Pub. Carnegie Inst., No. 48.
Tryon, R. C.
1934. "Comparative Psychology," Prentice-Hall Co., New York.
Winter, F. L.
1929. *Jour. Agr. Sci.*, Vol. 39.
Woodworth, C. M.
Illinois Agr. Exp. Station, Letter.
Also, See Annual Reports, Illinois Agr. Exp. Sta.

THE GENETICIST'S OBJECTIVES IN POULTRY IMPROVEMENT[1]

PROFESSOR F. B. HUTT
DEPARTMENT OF POULTRY HUSBANDRY, CORNELL UNIVERSITY

THE average producer of poultry and eggs would probably be satisfied if all members of his flock could mature in less than seven months and convert in a year about 95 pounds of feed into 200 eggs with an average weight of two ounces each, if mortality in that period were less than 10 per cent., if two thirds of the flock were worth keeping a second year and if his breeders could reproduce from 85 per cent. of their fertile eggs chicks capable of repeating the whole performance. To these desiderata may be added, as minor considerations, the special demands of the New York customer for white eggs, of Bostonians for brown ones, and similar local whims elsewhere, as well as the special needs of these poultrymen who produce the heavy fowls which poultry markets prefer.

These objectives, though modest, are much more easily stated than attained. In the first decade of the present century the economic needs of the poultryman were much simpler and the chief objective of those then seeking to breed better poultry was to produce fowls capable of laying more and still more eggs. This view-point has changed gradually with the realization that the market would pay a premium for large eggs, that enormous losses resulted annually from the failure to hatch of 35 per cent. or more of the eggs incubated, and, in later years, that mortality of pullets during their first laying year might run from 20 to 80 per cent.

Attainment of the geneticist's objectives in poultry improvement is complicated by two serious obstacles. In the first place nearly everything that he desires is dependent upon multiple factors rather than upon easily

[1] Read at a symposium on "Breeding to Meet Economic Needs" before the Genetics Society of America, American Association for the Advancement of Science, Indianapolis, Ind., December 30, 1937.

manipulated single genes. A slow process of progeny-testing is inevitable. Secondly, the attainment of any one of his several objectives is of little value without most of the others. A strain capable of high egg-production is undesirable if it can produce only 50-gram eggs for a market demanding a 24-ounce dozen. Of several different lines of Leghorns in the Cornell flock, that with the greatest viability during the past two years is utterly worthless economically because it has been so consistently bred for low fecundity that few of its members now produce as many as 100 eggs in a laying year. However, keeping before him the encouraging example of those plant breeders who have produced spring wheats not only resistant to many physiologic forms of stem rust, but also with stiff straw, high yield and good milling quality, the poultry geneticist struggles onward toward his *ultima Thule.*

To reach his objectives efficiently the geneticist really needs a great deal more information than is now available about those same objectives. This means that he must carry out two different programs. One of these seeks to sift the germplasm of his fowls as rapidly as possible through the sieve of the progeny test, to accumulate valuable genes and to eliminate those undesirable. The other program is concerned with the discovery of genes affecting the phenotypes desired and of means for determining more accurately the true genotype behind the observed performance. It seeks more light on the interaction of genes, on their manifold effects, their interactions with different environments and to find those outward and visible signs of inward and physiological grace to which the geneticist refers as an association between morphological and physiological characters.

In the following exposition of some of the more important objectives an attempt is made to show the way in which the straightforward work of progeny testing is interrelated with attempts to extend what is now known about the genetics of the fowl.

VIABILITY

While extensive losses occur during embryonic development and in the period of growth, nothing exceeds in importance at the present time the need of reducing by every means possible the prevailing high mortality among pullets successfully reared to breeding age.

Most of the figures quoted as measures of mortality in farm flocks are misleading because they omit from consideration birds culled as unprofitable producers, a class in which mortality is known to be much higher than among good layers. A better measure of mortality is obtained from the official laying tests, where birds from different sources are compared under uniform environment, and where all birds entered are kept for 51 weeks. Only carefully selected birds are sent by the breeders to these laying trials. They are kept in small flocks of 13 to 26 birds, under conditions in which epornithic outbreaks of disease are rare or absent, and where the social pressure on the weaker birds is much less than in large flocks.

Tabulation of results at thirteen such tests scattered in this country from Maine to Arizona and from Illinois to Florida shows that among 10,239 pullets mortality during 51 weeks in 1936–37 was 24.2 per cent. Losses in commercial flocks are considerably higher, and at few agricultural experiment stations does the mortality fall below 40 per cent. during the first laying year in unselected flocks not bred for viability.

The economic loss is not limited to the market value of the birds which die. Most of them are unprofitable layers for several months before they die (Harris, 1927, 1928). Loss of half the flock means that buildings and labor are not used with maximum efficiency and there is the additional cost of rearing more chickens each year than would be needed if mortality were negligible.

Of what do these birds die? Since the inception in 1931 of two New York State laying tests under the supervision of Cornell University, all birds dying at both have been sent to the New York State Veterinary College for diag-

nosis. An analysis by the writer of six-years' data from the annual reports of these tests reveals the distribution of causes of death shown in Table 1.

TABLE 1

CAUSES OF DEATH IN 1922 FOWLS (EXCLUDING 290 NOT DIAGNOSED) DYING AT TWO NEW YORK LAYING TESTS, OVER THE SIX YEARS 1931–37; 9,893 BIRDS ENTERED, MORTALITY 22.4 PER CENT

Causes of death	Incidence in deaths diagnosed
	per cent.
Neoplasms	38.4
Disorders of reproduction	25.3
Prolapse of oviduct	11.2
Kidney diseases	5.9
Impactions, alimentary tract	3.4
Internal hemorrhage	2.4
Other causes	13.4
	100.0

In this summary the term neoplasms includes tumors, neurolymphomatosis, leucosis, lymphocytoma and similar conditions. Disorders of reproduction include internal laying, ruptured yolks and impactions of the oviduct. Under "other causes" are included a miscellaneous assortment of conditions none of which by itself caused as much as two per cent. of the total mortality.

The significant point is that the conditions apart from "other causes" account for 87 per cent. of the mortality in 9,893 representative birds entered at these tests. The geneticist would feel less responsibility in the matter of reducing these losses if the orthodox techniques of the veterinarians, *i.e.*, sanitation, immunization and elimination of carriers and exposed birds, were as effective against those conditions now most serious as they have been in temporarily controlling fowl pox and pullorum disease. But this they are not. For none of the diseases causing 87 per cent. of the mortality at these tests are known to have any satisfactory preventive measures whatever, apart from breeding.

The rise in mortality is thought by some to have resulted in some way from a general rise in the productivity of fowls which is commonly believed to have occurred over the past twenty years. Conclusive proof for or against

this hypothesis has not yet been established. Unfortunately, the published data on the productivity of fowls in the laying tests of this country are not accurate measures of production because up to 1938 the published figures for average production have been based on the records of only the ten highest birds out of 13 entered per pen.[2]

However, figures from the English National Laying Trials at the Harper Adams Agricultural College show that, while the mortality rate there has risen steadily from 5.2 per cent. in 1925–26 to more than four times that figure in 1936–37, the average egg-production per bird has been slightly lower in the last seven years than in the period 1925–28. This does not prove anything except that a rise

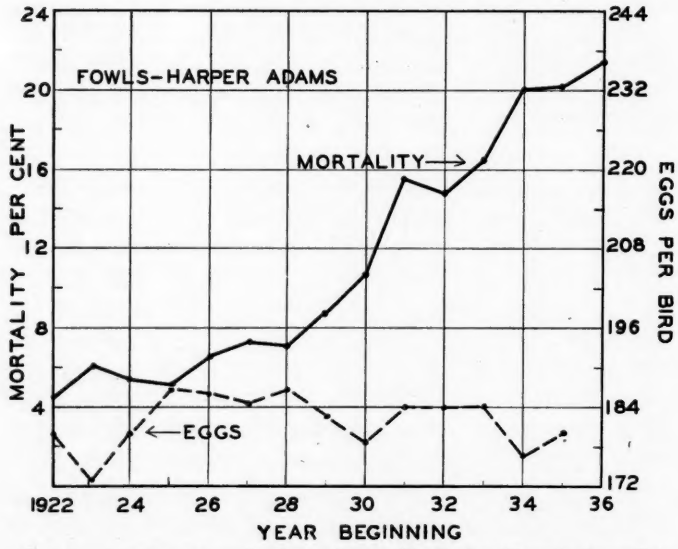


FIG. 1. Average egg production and mortality during 48 weeks for pullets entered in the Laying Trials at Harper Adams Agricultural College, Newport, Salop, England. The data are taken from volumes 8 to 22 of the *Harper Adams Utility Poultry Journal* and cover the laying years from 1922–23 to 1936–37.

[2] These efforts to gild the lily have fortunately been abandoned and standard laying tests will hereafter base their averages on the number of birds entered.

in mortality is not necessarily associated with higher egg yield by survivors. Autopsy records show that deaths in this English trial result from much the same causes as are chiefly responsible at the New York laying test. The rise in the frequency of neoplasms and of disorders associated with egg-laying is by no means a localized condition.

Fortunately evidence is accumulating to show that by proper breeding methods there can be established not only strains of fowls resistant to specific diseases, but also others with marked resistance to disease in general. In five generations of breeding for resistance to fowl typhoid, Lambert (1932) established a strain in which mortality during three weeks following inoculation was only 9.4 per cent., while in controls similarly treated 85 per cent. of the chicks died. Strains with marked resistance to *Salmonella pullorum* have been established by Roberts and Card (1935). While these experiments have been valuable in demonstrating the possibilities of genetic attack on poultry diseases, geneticists are still confronted with the task of producing strains of birds resistant under field conditions to the neoplasms and disorders of reproduction responsible for most of the mortality among adult birds.

How is this to be done? Controlled experiments in which successive generations are treated with standardized doses of a causative agent are desirable, but now almost impossible because of the wide divergence of opinions among poultry pathologists concerning the diagnosis, homologies and transmissibility of the various conditions here considered under the general heading of neoplasms. Pending the establishment of some order out of the existing chaos and the standardization of laboratory techniques, the geneticist must proceed under field conditions. This means careful progeny testing, maintenance of uniform environment for populations under test and such precautions against isolation and excessive sanitation as are necessary to ensure some exposure to whatever causative agents there may be.

Is such a breeding program likely to be effective? The

indications are that it will be. Extensive investigations have shown that in mice strains may be developed which differ significantly in susceptibility to lung tumors, others in susceptibility to mammary tumors, to leukemia or to neoplasms of other kinds. Similar data are not yet available for the fowl, partly because it is only in the last ten years that the seriousness of neoplasms in this species has been recognized. However, experiments seeking to establish genetic resistance to disease have recently been started in at least four state experiment stations and, although detailed reports have not yet been published, the indications thus far have been that mortality is markedly reduced even in the second selected generation. In 1936 the writer began breeding resistant and susceptible strains from a stock in which the mortality of adult pullets had exceeded 50 per cent. in each of the previous three years. In the second generation, hatched in 1937, the mortality among unselected pullets from 160 days of age up to November 28th was already 20 per cent. in the susceptible line but only 8 per cent. in the more resistant line. The latter figure is considerably below that for the corresponding period in the first generation. Both lots have been intermixed at random in the incubators and brooders, on the rearing range and in the laying pens.

The foregoing statement of the poultry geneticist's major problem raises many questions to which answers are not yet available. Are fowls resistant to neurolymphomatosis resistant also to lymphomatosis of the liver? If so, are they resistant to disorders of reproduction, or must we build a complex of many specific resistances, as seems probable from Gowen's (1933) demonstration that strains of mice highly resistant to one disease may be extremely susceptible to another? Lynch (1926) has shown that mice highly susceptible to lung tumors may be resistant to mammary tumors, and *vice versa*. Does this mean that specific resistances must be established for several different organs in the fowl? Under natural exposure will the spontaneous appearance of neoplasms and

other diseases prove adequate to make progeny testing effective, or will the incidence of these diseases be too low to permit differentiation of resistant and susceptible families? Is high egg-production partially incompatible with viability? These and many other problems require the cooperation of geneticists and pathologists for their solution.

Extension of Productive Life

While the most important immediate objective is that of keeping fowls alive for a year's production (*i.e.*, to about 18 months of age), from the long-time view-point extension of the productive life of fowls to three or four years or more is equally important, or more so. Among the survivors of the first laying year, productivity declines rapidly in succeeding years. Hall and Marble (1931) report that for 1,867 Leghorns kept for three laying years the production in their first, second and third years was 169, 146 and 124 eggs respectively. In their fifth year 450 survivors of these same birds, probably retained as superior stock, had an average production of only 96.5 eggs.

The majority of fowls are not kept for egg production beyond their first laying year. This means that the poultryman must bear annually the cost of renewing the flock, made excessively high by losses from infertile eggs, embryonic mortality and deaths during the period of growth.

A few fowls have shown exceptional capacity to maintain profitable production for several years. The most remarkable of these was a Leghorn reported by Hall (1938) which laid over 200 eggs annually (altogether 886) in her first four years, 188, 152, 175 and 114 eggs in succeeding years and died in her ninth with a total of 1,515 eggs to her credit. If the poultryman could only get a flock of such birds, and not have to renew his flock more than once in three or four years, the whole structure of the poultry industry would be altered and eggs could profitably be sold at much less than the prices now prevailing. This will not happen soon. The difficulty of

breeding for any character in which even the phenotype is unknown till the animal is from three to five years old is aggravated by the fact that the reproductive efficiency of fowls apparently declines somewhat after three or four years. These same conditions make difficult any application of the progeny test in breeding for sustained productivity and, if mass selection alone be utilized, progress toward the objectives will be slow. Greenwood (1937) has reported results of breeding which indicate that this very desirable character is amenable to selection and that the obstacles mentioned above are not insurmountable.

Information is urgently needed about the extent to which modification of certain environmental factors, now considered optimum, may influence longevity. The evidence of McCay (1934) and of McCay, Crowell and Maynard (1935) that length of life is extended in the rat by reducing the intake of feed suggests that the present common practice of keeping feed constantly before fowls at all stages of their lives may have an undesirable effect upon viability. There is no critical evidence to support the common belief among investigators in nutrition that the maximum rate of growth is optimum (except where early attainment of market weight is desired) and yet diets during growth are evaluated for farm animals according to their ability to promote rapid growth. Interesting in this connection are the experiments of Dove (1935) which indicate that neither the fastest nor the slowest rates of growth are compatible with maximum viability in the fowl, as measured by survival to one year of age, and that in between the extremes lies the optimum condition.

To encourage breeding for sustained production, less importance should be attached to records of first-year egg production, and more to the performance over a period of three or four years. Poultrymen, as well as breeders of dairy cattle, might well take a leaf from the book of the breeders of East Friesian cattle in Germany, who, according to Köppe (1936), have abandoned records of

performance for a registry of cows which have produced not less than 1000 kg. butter fat and five living calves by the end of their ninth year.

Efficient Reproduction

Estimates of the annual loss in the United States from failure of eggs to hatch vary from 20 to 35 million dollars. In the opinion of the writer this loss is not likely to be reduced by improvement in the environment during incubation or by changes in the mechanical operation of incubators. When these conditions are so satisfactory that 80 per cent. of the fertile eggs may hatch, and when some hens hatch over 95 per cent. of their fertile eggs, it seems likely that more benefit will result from improvement of the viability of the embryos. That this may depend somewhat upon improving the diets for the breeding stock is attested by the adverse effects upon hatchability when vitamins D, E or B_2 (riboflavin) are reduced to abnormally low levels in the diet of breeders, when essential minerals such as calcium and manganese are supplied at abnormal levels, or when a toxicant, such as selenium, is included in the diet.

It is probable, however, that the elimination of lethal genes from the breeding stock will prove the most effective means of raising hatchability. The marked rise in the viability of embryos and hatched chicks which results from suitable crosses (Warren, 1927) suggests that within any one breed lethal genes are to a considerable extent responsible for embryonic mortality. Five genes lethal to the homozygous embryo have thus far been demonstrated and it is certain that more will be found in the future. A recently-discovered sex-linked mutation, "naked," is lethal to about three quarters of the affected chicks (Hutt, 1938). Less is known about genes responsible for mortality after hatching but it seems probable that hereditary conditions, of which "congenital loco" (Knowlton, 1929) and congenital tremor (Hutt and Child, 1934) are examples, are to some extent responsible for the peak of mortality which occurs within two weeks of hatching.

Discovery of lethal genes and the studies necessary to establish criteria for their identification and differentiation, so that they may be eliminated from strains otherwise superior, constitute one of the more important objectives of the geneticist seeking to improve the domestic fowl.

EFFICIENT UTILIZATION OF FEED

Undoubtedly fowls differ, as other animals do, in the degrees of efficiency with which their feed is utilized for growth maintenance and production. The finding of Morris, Palmer and Kennedy (1933) that in nine generations of selective breeding they could establish two strains of rats, in one of which the utilization of feed was only 56 per cent. as efficient as in the other, suggests that similar breeding with fowls might have extremely valuable results. Such work has not yet been done with respect to total intake of feed but there is ample evidence of genetic bases for differential requirements of such limiting constituents of the diet as vitamins and certain minerals.

Geneticists and poultrymen have thus far failed to appreciate the significance of the fact that diets for fowls are now so formulated as to provide protection against shortage of any constituent known to be essential, no matter how extreme the variation among individuals may be with respect to their requirement of that constituent. In other words, the diet is made to protect the weakest and least fit bird in the flock. This is merely enhancing the usual tendency of domestication to permit much greater variation in a domesticated animal than is possible in the same species in nature.

The case may be illustrated by the conclusion of Ringrose and Norris (1936) that the minimum requirement of vitamin A for Leghorn chicks during the first eight weeks of life is about 150 U.S.P. units of that vitamin per 100 grams of feed. In their experiments 50 chicks receiving only two thirds of that amount were slightly lower in weight than those receiving 150 units, though not significantly so, but only one out of 50 exhibited any symptoms

of deficiency of vitamin A. While the protection of this one chick is undoubtedly desirable from the view-points of both the feed-manufacturer and the poultryman, it is obviously preferable from the ethnocentric standpoint to maintain the level of vitamin A at 100 units and pass along to posterity strains of fowls from which the extreme variants in the direction of unfitness have been systematically eliminated. It would seem, therefore, of considerable economic importance for the geneticist to establish breeding stock in which requirements of some of these essentials is reduced to a minimum, particularly with respect to expensive supplements like riboflavin and vitamin D.

This task is already partly done. Ample evidence has been adduced to prove that in ability to tolerate suboptimal levels of essential nutrients the Leghorns are markedly superior to the so-called heavy breeds. Nichita and his co-workers (1934) have shown that on diets deficient in vitamin B_1 adult Rhode Island Reds died of polyneuritis in from 9 to 21 days, while White Leghorns were extremely resistant, with some birds entirely unaffected after 78 days on such a diet. Lamoreux and Hutt (1937) using chicks of these same breeds and of Barred Rocks also found that Leghorns are markedly superior to heavy breeds in their ability to withstand a deficiency of this vitamin.

Slipped tendon of fowls, or perosis, is a condition in which at from 3 to 8 weeks the tibio-tarsal joint becomes enlarged and the tendon from the gastrocnemius muscle frequently slips out of the intercondylar groove. The fact that most cases are prevented by adding manganese to the diet indicates that deficiency of this element is largely responsible for the syndrome (Wilgus, Norris and Heuser, 1937). It is well known, however, that on ordinary diets White Leghorns seldom manifest the condition while in heavy breeds its occurrence is much more frequent. Serfontein and Payne (1934) found that perosis occurred in 14 per cent. of their Rhode Island Reds but in only 0.7 per cent. of the White Leghorns receiving the same diet. Moreover, within the more susceptible breed

those birds which had demonstrated resistance to perosis while on a deficient diet conducive to its causation produced offspring markedly resistant to the deficiency while breeders which, though susceptible, had survived produced chicks of which one half were also susceptible. There is evidently some genetic character associated with those constituting the White Leghorn breed which permits tolerance of a level of manganese too low to be adequate for heavy breeds, but it is significant that tolerance in the latter can be raised by selective breeding.

In the northern part of the United States and in Canada, a supplement of vitamin D is considered indispensable in winter, and, for confined birds, in summer also. It is usually supplied in cod liver oil. Olsson (1936) found that even as far north as southern Sweden, some hens are able to maintain satisfactory egg-production and hatchability without any supplement of vitamin D whatever. He aptly points out that if this attribute should prove to be hereditary, the selection of breeders should be made from flocks on diets with minimal rather than optimal levels of vitamin D.

These supplements and others are more important in the diet of the fowl than in larger animals because the storage in the smaller bird is more quickly exhausted, especially in those laying steadily. Some essentials, like vitamin B_1, are abundantly supplied in ordinary feeds, but others have to be added and some, like riboflavin, which is best obtained from milk, are among the most expensive constituents of the ration. It is time for geneticists to consider the selection of breeding stock from fowls whose variability from the mean requirements of these supplements is in the direction of tolerance of sub-optimal levels, and the elimination of those variants requiring the most protection in the diet.

Association of Morphological and Physiological Characters

Most animal breeders seek, whether consciously or otherwise, to discover some association between an easily

seen external character and a capacity to produce milk, eggs, meat, work or other things of economic value. A common poultryman's question of to-day "Which is the best breed?" was evidently equally prevalent nineteen centuries ago when Columella advised farmers that the most prolific fowls were likely to be red, with black wings, white ear lobes, five-toed and without spurs (in females). It is true that Columella would have considerable difficulty in proving his points but we do know of several very important associations of the kind to which he refers. Most of these are physiological differences between breeds.

White Leghorns are not only more resistant to some nutritional deficiencies but they also excel the heavy breeds in resistance to *Salmonella pullorum,* an attribute of great economic value. The writer has shown (Hutt, 1935) that White Leghorn chicks differ from Rhode Island Reds in the rate at which their temperatures rise in the first nine days after hatching and also in their erythrocyte and leukocyte counts. The Leghorns possess the sex-linked genes for rapid feathering, not found in many heavy breeds. This is a most interesting and exceptional case of a single gene determining a physiological character of considerable economic value.

As an offset to their superiority in many respects there is something about Leghorns which, according to Ackert (1935), makes them more susceptible than heavy breeds to parasitism by the nematode, *Ascaridia lineata.* Breeds also differ in capacity for reproduction. It is probable that the somewhat lower hatchability of eggs characteristic of White Wyandottes has been responsible for the decline in favor of this once popular breed.

In all of these cases, except that of rapid feathering, the physiological characters are ones that could not have been intentionally included in the establishment of the breeds, nor is it evident that they could be easily attained by artificial selection. Extension of the chromosome map may reveal that genetic linkages are in part responsible, and for that reason and others it is highly desirable to extend

our present knowledge of linkage in the fowl. By so doing we may expedite the incorporation of desirable physiological characters in our commercial breeds and the elimination of characters incompatible with economical productivity.

ADAPTATION TO ENVIRONMENT

Strains of the fowl with high viability in one region may be unsatisfactory in another. This is most marked when European or American stock is imported in tropical countries, but Hinshaw and Asmundson (1936) have shown that within the state of California turkeys susceptible to the contraction of pendulous crop, a condition with a genetic basis but much affected by environment, are much more likely to be affected in the hot valleys of the interior than along the coast. During an unusually hot week at Ithaca in July, 1936, the mortality among heavy breeds was about four times as great as among White Leghorns. Obviously genes are important in establishing stocks resistant to differing climatic conditions.

Apart from this the diseases prevalent in one locality may be somewhat different from those in another so that a strain with high viability in one region may be much less viable in another or even on another farm. For these reasons the geneticist's objectives may well include study of the relation of different environmental factors to viability and performance.

OTHER OBJECTIVES

It is understood that in striving to attain the desiderata discussed above the geneticist will not forget the importance of maintaining a profitable level of egg-production. In the opinion of the writer, if the other objectives are attained, higher flocks averages will follow, though the proportion of outstanding individuals may not be increased. It is doubtful if present techniques for analyzing characters dependent upon multiple factors are adequate to justify further attempts to assign a genetic basis for egg production, or for other quantitative characters, but

any new means of increasing the effectiveness of the progeny test are greatly to be desired.

Space prevents detailed discussion of other important objectives. Economic needs may well be met by a greater utilization of hybrid vigor and studies are desirable to determine to what extent that valuable condition may be effective in reducing mortality of embryos, chicks and adults or in extending productive life. Inbred strains are desirable for obtaining the maximum hybrid vigor, and, apart from immediate economic needs, they are urgently needed for studies in nutrition.

Even though improved strains should be attained without too much difficulty, the geneticist then has the problem of maintaining a number of quantitative characters in a sexually-reproducing organism, a feat much more difficult than the maintenance of improved varieties in most species of cultivated plants. Ways and means of doing this must be found.

Finally, the poultry geneticist must produce not only better fowls but also better poultrymen. Demonstration that some of the objectives discussed are not unattainable is more likely than anything else to encourage the better breeders in the task of establishing and maintaining superior germplasm in the domestic fowl.

LITERATURE CITED

Ackert, J. E.
1935. *Jour. Agr. Res.*, 50: 607–624.
Dove, W. F.
1935. Am. Nat., 69: 469–544.
Gowen, J. W.
1933. *Quart. Rev. Biol.*, 8: 338–347.
Greenwood, A. W.
1937. *Empire Jour. Exp. Agr.*, 5: 32–38.
Hall, G. O.
1938. *Jour. Hered.*, 29: 51–53.
Hall, G. O. and D. R. Marble
1931. *Poultry Sci.*, 10: 194–203.
Harris, J. A.
1927. *Poultry Sci.*, 6: 215–224.
1928. Am. Nat., 62: 508–531.

Hinshaw, W. R. and V. S. Asmundson
1936. *Jour. Am. Vet. Med. Assn.*, 88, N.S. 41: 154–165.
Hutt, F. B.
1935. AM. NAT., 69: 66–67.
1938. *Genetics*, 23: 152–153.
Hutt, F. B. and G. P. Child
1934. *Jour. Hered.*, 25: 341–350.
Köppe, A.
1936. *Deutsch. Landw. Tierzucht*, 40: 161–166 (cited from "Animal Breeding Abstracts," 4: 172).
Knowlton, F. L.
1929. *Oregon Agr. Exp. Sta. Bul.*, 253.
Lambert, W. V.
1932. *Jour. Immunol.*, 23: 229–240.
Lamoreux, W. F. and F. B. Hutt
1937. *Genetics*, 22: 198–199.
Lynch, C. J.
1926. *Jour. Exp. Med.*, 43: 339–355.
McCay, C. M.
1934. *Sci. Monthly*, 39: 405–414.
McCay, C. M., M. F. Crowell and L. A. Maynard
1935. *Jour. Nutrition*, 10: 63–79.
Morris, H. P., L. S. Palmer and C. Kennedy
1933. *Minn. Agr. Exp. Sta. Tech. Bul.*, 92.
Nichita, G. *et al.*
1934. *Ann. Inst. Nat. Zootech. de Roumanie*, 3: 35–109.
Olsson, N.
1936. *Nordisk Jordbrugsforskning*, 17–18: 185–218.
Ringrose, R. C. and L. C. Norris
1936. *Poultry Sci.*, 15: 390–396.
Roberts, E. and L. E. Card
1935. *Ill. Agr. Exp. Sta. Bul.*, 419.
Serfontein, P. J. and L. F. Payne
1934. *Poultry Sci.*, 13: 61–63.
Warren, D. C.
1927. *Poultry Sci.*, 7: 1–8.
Wilgus, H. S., L. C. Norris and G. F. Heuser
1937. *Jour. Nutrition*, 14: 155–167.

THE USE OF THE BACKCROSS IN CROP IMPROVEMENT[1]

DR. FRED N. BRIGGS

DEPARTMENT OF AGRONOMY, UNIVERSITY OF CALIFORNIA, DAVIS

THE science of genetics has been of great value to the plant breeder in that it has given him a clearer conception of his problems and a better understanding of the processes involved. However, there have been few cases where he has been able to make direct use of specific genetic principles to set up a scientific plant-breeding program. Such a program should enable him to predict accurately the nature of the variety which he will breed, or having produced such a variety, he could, if need should arise, repeat the breeding operations and obtain exactly the same variety. Within certain limits the backcross offers the plant breeder such an exact method.

The backcross, most useful in the transfer of specific characters, such as resistance to diseases and insects, is based on the simple fact that a heterozygous population backcrossed to either homozygous parent will become homozygous for the genotype of the recurrent parent. The proportion of homozygous individuals in any backcross generation is the same as would result from an equal number of selfed generations and may be expressed by the following well-known equation:

$$\text{Proportion of homozygosity} = \left(\frac{2^m - 1}{2^m}\right)^n$$

where n is the number of pairs of heterozygous factors and m is the number of generations of selfing or backcrossing as the case may be.

A theoretical consideration of the probable situation in a wheat cross will be of value. Available data indicate that such important characters as yield, quality and adaptation depend on multiple factors and that our best varie-

[1] Read at a symposium on "Breeding to Meet Economic Needs" before the Genetics Society of America, American Association for the Advancement of Science, Indianapolis, Ind., December 30, 1937.

ties in any locality have a fairly large number of favorable genes affecting these characters. It appears that many of these exert a relatively small effect, so that they come under the category of modifying factors. Consequently, they are hard to follow in hybrid populations with the result that the selection of plants with favorable combination of such genes is extremely uncertain.

Some of these problems can be illustrated best by considering a cross of Hope × White Federation from which we want to develop a variety having Hope's resistance to stem rust but at least equivalent to White Federation in yield, quality and adaptation. The following factors are known or assumed:

Hope	White Federation	Number of factors
Resistant to stem rust	Susceptible to stem rust	2
Awned	Awnless	1
Red kernel color	White kernel color	3
Yield poor	Excellent	9
Quality good	Good	2
Adaptation poor	Excellent	4
	Total	21

Twenty-one pairs of factors have been assumed which is one for each pair of chromosomes and not an excessive number. However, in the F_2 of such a cross the White Federation genotype would occur only once in 4,398,046,511,104 individuals. To grow such a population would require over 50,000,000 acres of land or about the area normally planted to wheat in the United States. Furthermore, in such a population there would be 2,097,152 homozygous genotypes, all different, and all but two potentially new varieties. To be sure, some of the other combinations might be equal to or superior to White Federation, a point which will be considered later.

It is apparent in the above cross that the 15 factors governing yield, quality and adaptation are the ones which complicate our plant breeding problem. If the above

hybrid is inbred for five generations, approximately half of the population will be homozygous and equally divided among the multitude of genotypes mentioned above. If five backcrosses are used the same degree of homozygosity is attained but only one homozygous genotype will be present, namely that of the recurrent parent. The rust resistant factors of Hope will have to be maintained by selection, but the other 19 factors are automatically taken care of by this method of breeding.

The ease with which the backcross can be used depends on a number of considerations. The simplest case is where the character to be transferred depends on a single gene about which the genetics is fully known. The frequency at which successive backcrosses may be made will depend on the ease with which the character to be transferred can be followed in hybrid populations. In many cases, considerable progress can be made by selection at the end of the first backcross. The F_1 of the above cross is capable of producing 2,095,152 different gametes, therefore the second inbred generation following the first backcross would exhibit considerable diversity. After the third or fourth backcross the material is so nearly like the recurrent parent that selection for characters other than the one being transferred is not very effective.

Even where the main consideration is the improvement in characters such as yield or quality a small amount of backcrossing might be employed to advantage especially if one parent of the hybrid is more desirable commercially than the other. One backcross might be made without the likelihood of losing any of the genes sought, provided a reasonable number of backcross seed is made. In the cross considered previously, the White Federation genotype will occur 2,097,152 times as frequently at the end of the first backcross as in a straight F_2, thus affecting a considerable shift in the population toward the better commercial parent.

Thus far, we have considered the use of the backcross only in self-fertilized crops. The question naturally arises as to its value in cross-fertilized crops, especially

those which lose vigor on inbreeding such as maize. The use of a heterozygous recurrent parent is equivalent to one generation of inbreeding. However, the proportion of homozygosity would not increase with successive backcrosses, beyond that reached by a single inbred generation. The most dependable use of the backcross will be to improve inbred lines in one or more characters. In such cases it will operate exactly the same as outlined above. Richey's (1927) plan for convergent improvement depends on backcrossing coupled with selection, involving a larger number of genes than is desirable for certain results.

Our actual experience with the backcross method in cereal improvement over a period of fifteen years involving a number of projects will be of interest. At various times since 1929, G. A. Wiebe, C. A. Suneson and W. B. Cartwright, all of the United States Department of Agriculture, have had an active part in this work. No attempt will be made to relate the activities of the various individuals except to point out that Mr. Cartwright has been active primarily on the Hessian fly project.

In 1922, Harlan and Pope (1922) pointed out the value of backcrosses in small grain breeding. That same year our first project was started with a view of transferring the bunt (*Tilletia tritici*) resistance of Martin to six of our already commercial or promising varieties of wheat. Six backcrosses were made between 1922 and 1932. After the first and third backcross the populations were inbred for three generations in order to get the factor for bunt resistance into the homozygous condition. Furthermore this gave an opportunity to select out the most desirable lines for further backcrossing. The material offered considerable opportunity for selection after the first backcross, but after the third most of these lines were so similar to the recurrent parent that they afforded little opportunity for further selection. Seventeen lines were chosen at this time for yield tests. On the basis of three years' data in replicated nursery rows, 10 lines representing four varietal types significantly out yielded their cor-

responding commercial parents, the other seven lines were not significantly different. A more thorough investigation of yield levels following varying numbers of backcrosses where random samples are used will be of interest.

After six backcrosses 70 or more lines from each cross were bulked to make up the new variety. One of the six original varieties, Hard Federation, did not achieve the commercial importance in California that it promised in 1922, and has not been increased here. Two varieties, White Federation and Baart, have been held in abeyance pending the incorporation of resistance to stem rust. The other three, Pacific Bluestem, Big Club and Sonora, were released for commercial production this year. These resistant varieties have retained the name of the original commercial parent with the year of release (in this case 37) appended to differentiate them from the susceptible sorts. This avoids any possible confusion among the growers and millers who are already familiar with the agronomic and milling qualities of these varieties. A comparison of yields obtained at Davis in 1937 follows:

	Av. yield in bu. per acre	Av. bushel weight
Pacific Bluestem	63.2	61.3
" " 37	61.2	61.3
Big Club	68.1	62.2
" " 37	68.1	62.4
Sonora	78.0	64.7
" 37	86.6	64.9

The yields are almost identical except for Sonora and they are not significantly different.

In 1930, seven other varieties were included in the bunt backcross project. Instead of using Martin directly for the resistant parent, lines derived from the third backcross of White Federation with Martin, which carried the Martin factor for resistance to this disease were used. They had many of the qualities desired for California wheats and therefore promised more rapid achievement of the desired end. These seven varieties will be ready

for commercial production in 1942. At that time then, bunt resistant strains of all commercially important varieties in this state will be available.

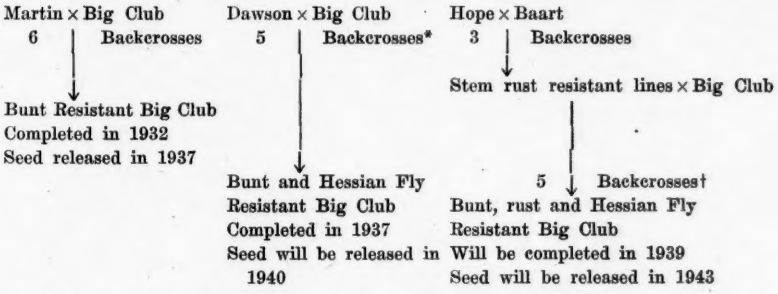
No difficulty has been encountered in obtaining lines similar to the commercial varieties or in maintaining a high degree of bunt resistance. Although Martin has been completely resistant to our collection of bunt over a period of 18 years, a number of the derived lines have picked up modifying factors which permit a slight infection when heavily inoculated. Experiments have shown that they will not produce enough inoculum to maintain the disease.

The incorporation of stem rust resistance in White Federation and Baart, our two most important varieties of wheat, was begun in 1930, using the Hope variety which is highly resistant to this disease. This backcross project was pushed along rapidly by growing two crops each year, which can be done in the field at Davis. Three backcrosses were made during successive generations. The material was inbred until homozygous for rust resistance before adding the last two backcrosses. Bunt resistant strains of White Federation and Baart were used in the last cross, thus bringing together resistance to the two diseases in these varieties. They are being increased and will be released for commercial production in 1939.

In the most comprehensive project undertaken thus far, bunt resistance, stem rust resistance and resistance to Hessian fly are being transferred to Big Club and Poso wheats. The three projects are being carried independently except that resistant strains are being used as the recurrent parent wherever available. An outline of this project for Big Club follows on p. 291.

The last cross has been made on the Hessian fly resistant project. Only two have been completed on the stem rust resistant program, so that bunt and Hessian fly resistant lines will be available for the last two crosses in the rust project.

Several projects are under way with barley which may be mentioned briefly. Resistance to mildew (*Erysiphe*



* Fourth and Fifth backcross made to bunt resistant Big Club.
† Second and Third backcross made to bunt resistant Big Club.
Fourth and Fifth backcross to bunt and Hessian fly resistant Big Club.

graminis), the awnless, hooded and naked characters are being transferred to Atlas barley. The low chromosome number (n = 7) in barley offers greater opportunity for linkage difficulties than in the case of wheat (n = 21). None of the above programs has been completed, but to date no apparent difficulties have been encountered.

A final interesting use of the backcross in our cereal program will be mentioned. It is almost impossible to distinguish three of our commercial barley varieties in the threshed sample. A long-haired rachilla is being transferred to Atlas, the most important of these varieties, which will easily distinguish it from the other two. Atlas then will be "trade marked."

Varieties developed by the backcross should furnish valuable data on a number of problems. For example it has not been possible to get exact information on the damage caused by stem rust of wheat. With two varieties, one susceptible and the other resistant but equal in other respects, a fairly accurate estimate of such damage should be obtained for various levels of infection.

Although there has been a sustained interest on the part of farmers in hooded and naked barleys, few if any varieties well adapted to conditions in the United States have been developed. By the use of the backcross, we should be able to determine if such varieties can be developed.

The value of awns on wheat long has been a subject of

debate, and the data thus far available are conflicting. The rôle of the awn in barley has not been established without a doubt, because plants in such studies have been subject to mutilation. By the use of the backcross, we should be able to determine if awnless varieties can be developed which are otherwise equal to awned varieties.

Finally, varieties derived in this manner should offer some evidence on Fisher's "Theory of Dominance." Within a few years, we will have the Martin factor for resistance to bunt in 12 varieties of California wheat in addition to five other fairly diverse varieties in which it has been found. Thus the same gene will be available in 17 quite different genetic environments. While it is definitely known that some of the modifying factors in wheat modify the homozygote, these varieties should offer material for investigating the effect of modifiers on the heterozygote as well.

The backcross is being extensively used here at California with other field crops, such as beans, cowpeas and inbred lines of corn. Likewise it has been found useful in truck crop improvement.

In closing, I would like to point out that in crops on which little improvement work has been done, notably many forage crops, there still remains a fertile field for selection and hybridization, such as has been practiced by plant breeders for many years. However, in such crops as wheat, which have been the object of improvement for many years, there are a large number of highly productive varieties. The immediate need for such crops is more dependable production. Much can be accomplished in this direction by adding disease and insect resistance to the best of these varieties. The backcross method is admirably suited for such an improvement program.

LITERATURE CITED

Harlan, H. V., and M. N. Pope
1922. *Jour. Heredity*, 13: 319.
Richey, F. D.
1927. AM. NAT., 61: 430–449.